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FATE OF THE C3H MILK INFLUENCE IN MICE OF STRAINS C AND C57 BLACK

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The foster nursing of low-mammary-tumor strains of mice by high-mammary-tumor-strain females produces mammary tumors in the fostered mice. All low-tumor strains tested have proved susceptible to the agent in the milk of high-tumor strains, but there is a difference in their degree of susceptibility. In general, strain C57 black mice are relatively resistant, whereas an experiment (*1*) revealed that strain C mice when suckled by high-tumor-strain C3H females showed a mammary-tumor incidence of 64 percent at an average age of 8.1 months. This high degree of susceptibility raised the question whether strain C mice can transmit the influence in their milk and if so whether it produces tumors in their progeny. It was found (*2*) that the C3H milk influence was transferred through at least two generations of strain C mice and that mammary tumors occurred in both generations.

This paper records in detail further results of the investigation on the fate of the strain C3H milk influence when introduced into strain C, and the results of another experiment in which the C3H milk influence was introduced into strain C57 black.

EXPERIMENTAL ANIMALS

All mice were raised in this laboratory. Those of strain C3H, a high-mammary-tumor strain, were used as a source of the milk influence. The derivation and tumor incidence of this substrain have been recorded (*3*).

Strain C mice were the descendants of a litter obtained in 1935 from Dr. George D. Snell, of the Roscoe B. Jackson Memorial Laboratory. The strain was derived from the Bagg albino stock and is listed as B alb C in the list of inbred strains published (*4*) by the Roscoe B. Jackson Memorial Laboratory. The original litter from Dr. Snell was in the F_{35} generation of inbreeding, and brother-sister matings only have been carried out in this laboratory. The strain is now in the F_{58} generation. Table 1 summarizes the occurrence of spontaneous mammary and pulmonary tumors in the first 19 completed generations of breeding females. The incidence of mammary tumors is 1.4 percent at an average age of 22.3 months.

Strain C57 black mice descended from a litter procured in 1939 from Dr. W. S. Murray, of the Springville Biological Station, Springville, N. Y. They are in the F_{14} generation of inbreeding since arrival in this laboratory, and the mammary-tumor incidence in breeding females is less than 1 percent.

All experimental animals lived under similar environmental conditions, ate Purina dog chow pellets, and drank tap water.

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EXPERIMENTAL PROCEDURE AND RESULTS

FATE OF THE STRAIN C3H MILK INFLUENCE IN STRAIN C MICE

Preliminary findings in the first 3 generations have been published (*2*). The experiment was started by transferring 5 litters of strain C mice within 17 hours after birth to strain C3H foster mothers. When the young were weaned, they were called the F generation and were the only strain C mice in this study which obtained milk from or had any contact with strain C3H mice. There were 20 females and 8 males in this generation, and all were from the F_{47} or F_{48} generation of the strain C colony. Their mothers, each of which bore 3 litters, died free of mammary tumor at an average age of 22.5 months. Of their 5 strain C3H foster mothers, 2 developed mammary tumor when 5 months old and 3 died without tumor when 6 months old.

To test for transmission of the milk influence, all 20 female F mice were mated to brothers, and subsequent generations were procured by brother-to-sister breeding only. Each F-generation mouse bore 3 litters. One failed to raise a litter; the remaining 19 each raised 1 litter, and 2 litters were killed within 24 hours after birth. Three nursed their first litter, 11 the second, and 5 the third. Each litter contained mice of both sexes, and 61 females were raised. These were the F_1-generation mice.

The F_1 females bore 1, 2, or 3 litters, but none nursed more than 1 litter; all others were killed shortly after birth. Eight did not raise a litter, 5 nursed their first litter, 37 the second, and 11 the third. Fifty-two suckled litters consisting of both sexes and containing 159 females, which were called F_2-generation mice.

The F_2 females were kept as 3 groups: virgins, and those bearing 1 or 2 litters. None bearing 1 litter nursed its young, which were killed within 24 hours after birth. Those bearing 2 litters were permitted to raise their first litter only; the second litter was killed shortly after birth. Twenty of the forty mice in the 2-litter group lost their first litter, and the others reared litters containing both sexes. Thus, 46 females of the F_3 generation were procured.

Twelve females of the F_3 generation each bore 1 litter which was killed soon after birth. The remaining 34 each bore 2 litters but were permitted to nurse their first litter only. All raised their young; only 1 litter contained males only. A total of 103 female mice of the F_4 generation was obtained.

At this time a procedure was adopted to give uniform test animals for transmission of the milk influence and to keep the number of mice within practical limits. The breeding routine started in the F_2 generation was continued. Each female bore 2 litters but nursed only the first to produce mice of the next generation. Offspring from 1 female of each litter were mated. For example, the 103 mice of the F_4 generation were born to 33 females which belonged to 19 litters. The young of only 1 female in each litter

TABLE 1.—*Occurrence of spontaneous pulmonary and mammary tumors in 19 generations of breeding strain C female mice*

Mice	Age in months																							Total	Average age at death	Tumor incidence
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29			
																											Mo.	*Pct.*
Number examined	3	7	3	5	1	5	11	12	26	27	18	26	29	30	22	36	62	48	49	55	12	7	3	2	3	502	19	
Number with pulmonary tumor							1	1	5	3	1	3	5	4	6	10	21	17	16	21	5	6	1	1	2	129		25.7
Number developing mammary tumor															1	1	2	1		1					1	7		1.4

TABLE 2.—*Occurrence of mammary tumors in fostered strain C female mice and in 11 generations of their descendants*

[Numerator = mice developing tumors. Denominator = mice dying without tumors]

Generation	Number of litters born	Number of mice	Age in months																				Total	Tumor incidence	Average tumor age	Average age at death without tumor
			4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24				
																								Pct.	*Mo.*	*Mo.*
F	3	20	---	1/0	3/0	---	----	2/0	3/0	1/0	3/0	1/0	---	---	0/2	---	---	---	---	0/1	---	---	0/3	14/6	70	9.3	20.8
F_1	1	4	1/0	---	---	---	---	---	---	---	1/0	---	1/0	---	---	---	---	---	---	---	0/1	---	---	3/1	75	10.0	22.0
F_1	2	9	---	---	1/0	3/0	3/0	1/0	---	---	---	1/0	---	---	---	---	---	---	---	---	---	---	---	9/0	100	8.1	---
F_1	3	48	---	1/0	1/0	9/0	9/1	9/0	5/1	---	4/0	---	---	---	1/0	---	0/1	1/0	---	0/1	---	0/3	0/1	40/8	83	9.0	18.7
F_2	0	26	0/1	---	---	---	---	1/0	1/0	---	---	0/1	3/2	2/0	1/0	1/0	1/1	---	0/2	1/1	2/4	0/1	---	13/13	50	15.9	18.1
F_2	1	93	---	1/0	2/0	6/1	5/0	10/1	11/0	8/0	10/0	7/0	1/0	1/0	1/0	2/0	1/0	1/1	0/1	1/0	1/7	1/12	---	70/23	75	11.1	21.1
F_2	2	40	---	2/0	2/0	6/0	10/0	4/0	6/1	3/0	---	---	1/0	---	---	---	---	---	1/2	---	---	0/1	0/1	35/5	87	8.7	19.4
F_3	1	12	---	---	---	---	---	1/0	---	1/0	1/0	2/0	1/0	1/0	1/0	1/0	---	---	0/3	---	---	---	---	9/3	75	13.3	20.0
F_3	2	34	---	---	1/0	2/0	5/0	6/0	4/0	3/0	4/0	3/0	2/0	0/1	---	---	0/1	---	0/1	0/1	---	---	---	30/4	88	10.6	18.5
F_4	2	40	---	---	1/0	3/0	5/0	8/0	8/0	5/0	2/0	3/0	2/0	---	---	---	---	0/1	---	---	---	---	0/2	37/3	92	9.9	22.3
F_5	2	41	---	---	2/0	4/0	6/0	4/0	8/0	6/0	2/0	3/0	---	---	1/0	---	---	0/1	---	---	0/1	0/1	0/2	36/5	87	9.7	22.4
F_6	2	36	1/0	---	2/0	1/0	7/0	1/0	5/0	6/1	3/0	4/0	---	1/0	1/0	1/0	---	---	---	---	0/1	0/1	---	33/3	91	10.3	18.6
F_7	2	35	---	1/0	2/0	2/0	5/0	3/0	5/0	8/0	3/0	1/0	---	2/0	---	---	---	1/0	---	0/1	0/1	---	---	33/2	94	10.1	21.5
F_8	2	29	---	---	3/0	4/0	1/0	10/0	---	2/0	2/0	3/0	1/0	---	2/0	---	0/1	---	---	---	---	---	---	28/1	96	9.8	18.0
F_9	2	22	---	---	1/0	2/0	2/0	1/0	2/0	4/0	5/0	---	2/0	1/0	---	---	1/0	---	0/1	---	---	---	---	21/1	95	10.9	20.0
F_{10}	1	16	---	---	---	1/0	1/0	1/0	1/0	---	4/0	2/1	1/0	---	---	1/0	---	1/1	0/1	---	---	---	---	13/3	81	12.1	17.3
F_{11}	1 to 3	15	---	---	---	1/0	----	1/0	1/0	3/0	3/0	1/0	---	4/0	---	---	---	0/1	---	---	---	---	---	14/1	93	12.0	19.0

were bred. This procedure established 19 lines of mice. If all females of any litter failed to raise their first litter or if they reared males only, that line was not continued. At no time previous to or after the beginning of this routine was any selection made in respect to tumor occurrence. The only selection was to choose, if possible, a litter consisting of 3 females and 2 males to propagate a line. Up to and after this time, all mice were mated when 2 to 4 months of age.

The routine was followed until the F_{10} generation was procured, and 10 generations of mice were tested for transmission of the strain C3H milk influence through strain C mice. Then 2 lines of the F_{10} generation were selected for further propagation of the colony. On January 1, 1945, these mice were in the F_{15} generation of inbreeding.

The results in 12 completed generations are presented in table 2. Mammary tumors arose in 14 of the fostered strain C mice of the F generation, which were the only mice to ingest strain C3H milk. Their average tumor age was 9.3 months; the remaining 6 died without mammary tumor at an average age of 20.8 months. Strain C3H milk influence raised the mammary-tumor incidence in strain C mice from 1.4 (table 1) to 70 percent. Of the 502 strain C breeding females included in table 1, 189 bore 3 litters, and 4 developed breast tumor.

Mammary tumors arose in 85 percent of the F_1 generation. Occurrence of tumors in these mice was independent of the appearance of tumor in their mothers. Forty-one of the F_1 mice were born to 13 mothers of the F generation, and 36 (88 percent) developed tumor at an average age of 8.9 months. Twenty were born to the 6 F-generation females that remained tumor-free, and 16 (80 percent) developed tumor at an average age of 8.9 months. This was also true for subsequent generations and is mentioned in connection with the histories of the various lines.

Table 2 shows that the C3H milk influence was transferred through 12 generations of strain C mice and transformed the latter into a high-mammary-tumor strain. This fact indicates that the milk influence of strain C3H is capable of reproduction in or propagation by strain C mice.

Table 2 also shows the influence of breeding upon the appearance of breast tumors in strain C mice that contain the milk influence. The nonbreeding mice

of the F_2 generation exhibited a tumor incidence of 50 percent at an average age of 15.9 months; those bearing 1 litter in the F_1, F_2, F_3, and F_{10} generations showed an incidence of 75 or 81 percent at average ages of 10, 11.1, 13.3, and 12.1 months, respectively, whereas those of 9 generations bearing 2 litters had incidences ranging from 87 to 100 percent at average ages from 8.1 to 10.9 months. Mice of the F and F_1 generations that bore 3 litters gave results suggesting that bearing more than 2 litters does not raise the incidence of tumors but may lower the average age at which tumors appear.

All the F-generation mice developing tumors did so before they were 14 months old, and 6 died tumor-free at the age of 16, 21, or 24 months. Of 43 mice in the F to F_{10} generations bearing 2 or 3 litters and living to be over 15 months of age, 10 (23 percent) developed tumors. Only 4 (11 percent) of the 35 that lived to be over 17 months old developed tumors. This implies that some mice of strain C may be very resistant to the activity of the milk influence or hormonal stimulation or both, and the strain may thus provide suitable experimental animals for determining nongenetic factors influencing tumor occurrence.

The apparent resistance of some strain C mice to the milk influence raised the question whether it disappeared or became inactive in certain lines. As stated before, no effort toward selection was made during the course of the study, and the records contain only two instances which could be interpreted as suggesting a disappearance or quenching of the influence. They are summarized as follows:

(1) A mouse of the F generation bore three litters and died tumor-free when 24 months old. She nursed two daughters, both of which gave birth to three litters. One died without a tumor when 23 months old. She nursed five daughters, each of which bore one litter that was killed soon after birth. All five lived to be 23 months old, and none developed a tumor.

(2) An F_1 mouse gave birth to three litters and died without tumor at 21 months. One of her offspring bore two litters and died tumor-free at 20 months. She raised one daughter that gave birth to two litters, one of which was raised but did not contain a male. She died when 18 months old and did not have a tumor.

In both instances, two generations of breeding mice descended from a female that was exposed to the milk influence, but mammary tumor did not occur in any animal. Further work is needed to prove that some strain C mice are able to destroy the milk influence.

In addition to the information furnished by the mice of the F and F_1 generations, there was abundant evidence that strain C mice can transmit the milk influence and not develop a breast tumor. This evidence was supplied by the 19 lines of progeny derived from the litters of the F_3 generation. Four such lines are given in table 3. Lines C and G are offered as representative lines, and D and I because they constitute the present colony. In line C, the F-generation ancestor which obtained the strain C3H influence did not develop a tumor, but tumors arose in the next 3 generations of descendants. The F_4 mouse interrupted the tumor sequence and died at 24 months, but the next 5 generations in the line all developed mammary tumors. Line D, similar to

TABLE 3.—*Four typical lines of progeny from fostered strain C females*

Generation	Line C		Line D		Line G		Line I	
	Mouse No.	Age	Mouse No.	Age	Mouse No.	Age	Mouse No.	Age
		Mo.		*Mo.*		*Mo.*		*Mo.*
F	3	−21	14	+10	16	+9	9	+6
F_1	24	+8	54	+9	46	+7	33	+7
F_2	115	+8	108	+8	99	+6	87	+8
F_3	259	+8	286	+7	278	+6	252	+12
F_4	310	−24	331	+10	293	+14	324	+11
F_5	351	+8	374	+12	337	−24	369	+8
F_6	393	+11	411	+13	379	+17	406	+11
F_7	421	+7	425	+8	434	+10	440	+12
F_8	451	+11	464	+9	474	+14	467	+9
F_9	487	+8	483	+9	497	+12	484	−20
F_{10}	.....	.....	519	+14	.....	.....	522	+12
F_{11}	.....	.....	539	+10	.....	.....	540	+11
F_{12}	.....	.....	546	+10	.....	.....	558	+14
F_{13}	.....	.....	568	L13	.....	.....	572	L12

+ denotes occurrence of mammary tumor.
− denotes death without mammary tumor.
L denotes living and free of tumor.

several others, contained tumor-bearing mice in every generation.

During the course of the study an experiment was performed to ascertain whether the strain C3H milk influence was decreasing in amount or activity during passage through strain C animals. Female hybrids derived by mating strain I females to C3H males were used as test animals since they were known to be susceptible to the milk influence. Within 17 hours after birth, part of each litter of hybrids was given to a C3H female and part to an F_5-generation female of the fostered strain C colony. Some litters remained with their C3H or C foster mothers for 48 hours and were then returned to their strain I mothers for the remainder of the nursing period; others remained with their foster mothers throughout the nursing period. Of 27 hybrids that nursed upon C females for 48 hours, 96 percent developed mammary tumors at an average age of 8.7 months, whereas of 44 litter mates that nursed upon C3H females for 48 hours, 95 percent developed tumors at an average age of 7.5 months. Twenty-four hybrids raised by strain C foster mothers all developed tumors at an average age of 8.4 months, and 9 litter mates raised by strain C3H foster mothers all developed tumors at an average age of 8 months.

The outcome of this experiment suggests that the C3H milk influence after passage through six generations of C mice had not decreased appreciably in amount or activity. Administration of measured quantities of C3H or C milk to the hybrids would be a better test for relative activity.

FATE OF THE STRAIN C3H MILK INFLUENCE IN STRAIN C57 BLACK MICE

When it was apparent that the C3H milk influence was transferred through successive generations of strain C mice, it was decided to ascertain whether it would also undergo passage through C57 black mice. A litter of mice of the C57 black strain consisting of two females and one male was given, immediately after birth, to a strain C3H foster mother. These were the only C57 black animals in this study that obtained C3H milk. Their mother died tumor-free at 14 months, and their foster mother developed a mammary tumor when 7 months of age.

When the fostered C57 black mice were 2 months old, they were mated brother to sister, and all subsequent generations were obtained in the same manner. Since C57 black mice were known to be more resistant to the milk influence than were those of strain C, it was considered advisable to use I $\times$ C3H hybrid females as test animals for the presence of the milk influence. This procedure would reveal whether the milk influence passed through successive generations of C57 black females without producing tumors in them. Consequently, to every litter of fostered C57 blacks and to some litters in each generation of their descendants, newborn I $\times$ C3H hybrids were added. When 2 to 3 months old, each fostered hybrid bore one litter which was killed soon after birth. The results of this study are given in table 4.

Both fostered mice of the F generation died without mammary tumor at 22 months. Each bore 4 litters, 1 raised all and 1 lost her last litter. They fostered 13 hybrids, of which 12 developed mammary tumor at an average age of 10 months. Two of these hybrids nursed with the first litter born to the C57 black mice, and both developed tumor at 8 months; of 3 that nursed with the second litters, 2 developed tumors at 8 and 11 months and 1 died without tumor at 28 months; 12 that nursed with the third litters all developed tumors at an average age of 12 months; 2 nursed with the fourth litter, and both developed tumor when 5 and 6 months old. The black mice suckled by a C3H foster mother transmitted the influence in their milk.

The F_1 generation consisted of 9 females. One did not give birth to a litter, and the others bore from 1 to 3 litters. These mice fostered 11 hybrids, none of which developed mammary tumor. Two more generations were obtained, and there were no breast

TABLE 4.—*Occurrence of mammary tumors in descendants of fostered C57 black mice and in female I×C3H hybrids suckled by them*

Generation	C57 black					Fostered I×C3H hybrids			
	Number of litters born	Number of mice	Number developing mammary tumor	Average age at death without tumor	Number suckling I×C3H hybrids	Number of mice	Number developing mammary tumor	Average tumor age	Average age at death without tumor
				Months				*Months*	*Months*
F	4	2	0	22	2	13	12	10	28
F_1	0	1	0	20	0	0	----	----	----
F_1	1	4	0	19	2	4	0	----	22
F_1	2	2	0	19	2	4	0	----	25
F_1	3	2	1	21	1	3	0	----	25
F_2	1	5	0	21	4	8	0	----	24
F_2	2	3	0	12	3	9	0	----	25
F_3	0	8	0	24	0	0	0	----	----
F_3	1	8	0	22	7	10	0	----	23
F_3	2	2	0	26	0	0	0	----	----
F_4	0	1	0	25	0	0	0	----	----
F_4	1	3	0	21	3	6	0	----	21
F_4	3	2	0	14	2	3	0	----	17

tumors in any of the C57 blacks or in the hybrids nursed by them. It is clear that in contrast with strain C mice the C57 black females did not transmit the C3H milk influence through successive generations.

A mammary tumor arose in one C57 black of the F_1 generation. This animal was in the first litter born to an F-generation mouse. A litter mate that bore three litters died tumor-free at 21 months. The I × C3H hybrids added to this litter died. However, all five others fostered by the F-generation mother in two subsequent litters developed tumors. Apparently the F_1 generation mouse obtained the milk influence from her mother. She bore three litters and raised two blacks and three hybrids. The hybrids lived to be 19, 27, and 29 months old and did not develop tumor. The two black offspring raised three litters and fostered six hybrids, none of which had a tumor at death. These data indicate that if the milk influence produced the tumor in the F_1 mouse, the influence was not transmitted in her milk. A test for the presence of the influence in the tumor was not made.

DISCUSSION

The fate of the milk influence when introduced into low-mammary-tumor strains of mice has not received much attention. Bittner (*5*) noted that low-tumor-strain females fostered by high-tumor-strain mice could transmit the influence in their milk although they did not develop breast tumors, and this was amply confirmed in the present investigations. Moreover, when the C3H milk influence was introduced into C females, it was transmitted through at least 12 successive generations of inbred mice and transformed strain C into a high-mammary-tumor strain. The strain is more susceptible to tumor development than is strain A since 50 percent develop tumor if kept as virgins, and 75 percent do so if they give birth to 1 litter. In this laboratory less than 5 percent of virgin strain A mice give rise to mammary tumors, and less than 25 percent do so if they bear 1 litter only. Strain C3H is more susceptible than the new strain since virtually all virgin or breeding mice of strain C3H develop tumors.

Under the conditions reported here, the C3H milk influence was not transferred to successive generations of C57 black mice. This is not interpreted as showing that all mice of this strain cannot propagate the influence. Bagg and Jacksen (*6*) subjected strain C57 black to intensive hormonal stimulation and derived a strain that gave a high incidence of breast tumors. To date, a test for the presence of the milk influence in Bagg's high-tumor line has not been reported, and Little and

Pearson (7) could not confirm the results with their C57 black females.

Fekete and Little (8) transferred fertilized ova from C57 black mice to the uteri of high-mammary-tumor-strain dba mice. Mice born from the transferred ova were suckled by mothers which gave birth to them. Among 14 mice obtained in this manner, 4 did not have offspring; and of the remaining 10, 5 developed mammary tumor. Three of those developing tumors and all five of those that did not had from 1 to 3 generations of descendants, and mammary tumors arose in some mice of each generation in all lines. Thus, as Fekete and Little state, "transferred-ova animals differed from C57 black animals by transmitting a tendency to form carcinoma of the mammary gland in their offspring." The C57 black mice in their experiment developed in the uteri of strain dba mice, and this may be at least partially responsible for their results.

Miller and Pybus (9) reported the production of breast tumors in a substrain of CBA that had nursed upon a high-tumor strain, and the tumor incidence in the first two generations of descendants of the fostered mice remained high. CBA mice may resemble strain C mice in this respect.

It is possible that the milk influence was not involved in the origin of all the breast tumors in the C57 black mice of Bagg as well as those of Fekete and Little. If it is assumed that the milk influence was responsible, then their results, together with those recorded herein, indicate that some C57 black mice transmit the influence in their milk and others do not. This difference could be explained on the basis of variations between substrains or individual animals within a substrain. In the present study, only two C57 black litter mates obtained the milk influence. Perhaps the use of more litters would reveal that some lines transmit the influence beyond those receiving it from the C3H females. There is sufficient evidence, however, to conclude that transmission of the influence is less likely to occur in C57 black animals than in those of strain C.

If the influence is capable of reproduction in or propagation by all strains of mice, then the occurrence of mammary tumors in inbred mice is the result of a fortuitous exposure of mammary tissues to an extrinsic agent. The findings reported here indicate that in contrast with strain C all the C57 black animals are not able to transmit the influence through successive generations. This fact suggests that the production of a high-breast-tumor-strain of inbred mice depends not only upon the presence of the milk influence but also upon a genetic constitution which enables the mice to propagate and transmit the agent in their milk.

SUMMARY

Five litters of low-mammary-tumor-strain C mice were suckled by high-mammary-tumor-strain C3H females. Every strain C mouse transmitted the influence to her offspring, and this transmission continued for nine generations of inbreeding. Each generation showed a high incidence of mammary tumors in breeding females. Two lines are now in the F_{15} generation of inbreeding and continue to show a high tumor incidence. A single exposure of C mice to C3H milk influence transformed strain C into a high-mammary-tumor strain.

One litter of low-mammary-tumor strain C57 black mice was suckled by a C3H female. The fostered C57 black animals transmitted the influence to their offspring in sufficient amounts to produce mammary tumors in susceptible I $\times$ C3H hybrids; but only one of nine C57 black offspring developed a tumor, and none transmitted sufficient influence in her milk to produce tumors in I $\times$ C3H hybrids. Two additional generations of C57 black animals also failed to show any evidence of transmission of the influence.

Apparently the genetic constitution of inbred mice determines not only their susceptibility to the influence but also their ability to transmit or propagate the influence.

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RELATION OF MILK INFLUENCE TO MAMMARY TUMORS OF HYBRID MICE

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During an experiment (*1*) in which inbred mice and their hybrids received injections of *o*-aminoazotoluene, it was noted that spontaneous mammary tumors arose in untreated control females derived by mating strain C females to strain C3H males. There were 20 mice in the control group, and 4 developed typical mammary tumors when 5, 6, 9, and 13 months of age. It was known that strain C females revealed a high incidence of mammary tumors if they ingested milk of high-mammary-tumor-strain C3H females (*2*) and transferred the milk influence through successive generations (*3*).

The question arose whether the milk influence was involved in the occurrence of tumors in the (C × C3H)F_1 hybrids. It was conceivable that the milk of a few or all strain C females might contain a small amount of the influence which would not produce tumors in their strain C offspring but would do so in hybrids which are more susceptible than the strain C mice. If the milk influence is capable of reproduction in or propagation by susceptible mice, it appeared unlikely that it would not increase in amount or activity in a susceptible strain; but Bittner (*4, 5*) had published a finding suggesting a gradual diminution instead of increase in the amount of influence in successive generations of susceptible animals. He kept high-mammary-tumor-strain A mice with their mothers a maximum of 24 hours after birth before transferring them to low-mammary-tumor-strain C57 black foster mothers. Six of these fostered A mice developed tumors, and four generations of their descendants showed tumor incidences of 80, 64, 50, and 37 percent.

An experiment was performed to confirm the earlier observation with (C × C3H) F_1 hybrids and to ascertain whether the milk influence was involved in the occurrence of the tumors.

EXPERIMENTAL PROCEDURE

All mice were raised in this laboratory. They were kept under similar environmental conditions with an unlimited supply of tap water and Purina dog chow pellets.

The experiment was started during March 1941 by mating strain C females to strain C3H males. The derivation and mammary-tumor incidences of both strains have been reported (*6, 7*). Breeding strain C females show a mammary-tumor incidence of 1.4 percent. Nineteen strain C females and eight strain C3H males were mated, and every resultant litter contained female mice. When the litters were born, the males were discarded, and to each litter were added one to three female (I × C3H) F_1 hybrids. These mice were known to be very susceptible to the milk influence (*7, 8, 9*) and served as test animals for its presence in the milk of the strain C animals.

After the hybrids were weaned, they were kept until 2 months old and mated. Each mouse bore 3 litters in rapid succession, that is, they were kept with males continuously, and the litters were killed soon after birth. There were 75 (C × C3H) F_1 hybrids and 36 (I × C3H) F_1 hybrids obtained in this manner.

Histologic sections were prepared from each tumor and were diagnosed by Dr. Thelma B. Dunn, of the National Cancer Institute.

RESULTS

The findings are summarized in table 1. Thirty-nine of the seventy-five (C × C3H) F_1 hybrids and one of the thirty-six (I × C3H) F_1 hybrids developed typical mammary-gland tumors. Nine others grew tumors which were classified as atypical mammary growths because they consisted mostly

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TABLE 1.—*Occurrence of spontaneous mammary tumors in offspring of strain C females and strain C3H males and in (I×C3H) F_1 hybrids fostered by the strain C females*

[Numerators=Number of mice developing tumor. Denominators=Number of mice dying without tumor]

Derivation	Number of mice	Age in months																		Totals	Tumor incidence	Average age at which tumors occurred	Average age at death without tumor
		8	13	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				
																					Pct.	*Mo.*	*Mo.*
C×C3H	75	2/0	1/1	1/0	2/1	1/0	1/1	1/1	2/3	4/3	2/1	6/3	3/2	1/6	2/4	7/2	1/3	2/3	0/2	39/36	52	22	24
I×C3H	36	----	----	----	----	----	0/1	0/2	0/2	0/1	0/2	0/1	0/1	0/5	0/2	0/6	1/5	0/5	0/2	1/35	3	28	25

of mammary-tissue changes described by Haaland (*10*) as occurring in old female mice. However, they usually contained some areas of definite malignant change. Seven atypical growths occurred in (C × C3H) F_1 hybrids: One at 18, one at 20, two at 21, one at 23, and two at 27 months of age; and two arose in (I × C3H) F_1 hybrids 26 and 27 months old. If these tumors are included, the (C × C3H) F_1 mice showed a tumor incidence of 60, and the (I × C3H) F_1 of 8 percent.

The three tumors in (I × C3H) F_1 hybrids appeared in mice suckled by three strain C females. Each hybrid had a litter mate that nursed on the same C female and did not develop tumor. They died when 30, 23, and 28 months old.

TABLE 2.—*Distribution of tumors in (C×C3H) F_1 hybrids and in fostered (I×C3H) F_1 hybrids according to litter*

Mother No.	Number of litter	(C × C3H) F_1 hybrids		Fostered (I × C3H) F_1 hybrids	
		Number of mice	Number developing tumor	Number of mice	Number developing tumor
353	4	5	3	1	0
363	4	6	3	2	0
366	3	4	2	2	0
460	3	3	2	2	0
468	4	4	2	1	0
475	3	3	1	2	0
476	3	3	2	1	0
478	3	3	2	2	1
483	2	4	0	2	0
485	3	6	5	3	0
496	3	4	3	2	0
497	3	5	3	2	0
503	2	4	2	2	0
510	2	3	2	3	0
517	2	2	0	2	0
523	2	5	1	1	0
526	2	3	2	2	0
546	2	5	1	2	0
550	2	3	3	2	0

Distribution of the (C × C3H) F_1 and (I × C3H) F_1 hybrids according to litters is shown in table 2. The first column gives the colony number of the strain C mother, and the second, the number of the litter she bore in this experiment. The offspring in only two litters failed to develop tumor. The tendency toward tumor development in the hybrids was not limited to certain C mothers or C3H fathers.

Eighteen of the strain C females lived to an average age of 20 months without developing mammary tumor. One (No. 468) developed a typical mammary tumor when 21 months old. She raised four (C × C3H) F_1 hybrids of which two developed tumor when 8 months old and two died tumor-free at 19 and 21 months of age. She fostered one (I × C3H) F_1 hybrid which died without tumor at the age of 27 months. Except for the tumors in her (C × C3H) F_1 offspring, which were the first tumors to appear in this experiment, there was nothing in the history of her litter or fostered (I × C3H) F_1 hybrid to indicate that her milk contained the influence.

Efforts were made to detect the influence in tumors arising in (C×C3H)F_1 by oral administration of tumor extract to (I×C3H)F_1 hybrids. All tumor extracts were prepared by Dr. W. R. Bryan (*11*), of the National Cancer Institute. Extracts were made from 5 tumors which appeared in mice

8, 18, 20, and 21 months old; 2 were from 20-month-old mice. The growth in the 8-month-old mouse appeared in an offspring of strain C female No. 468, mentioned in the preceding paragraph. The tumors were removed aseptically, weighed, ground with sand, and made up to a 20- or 25-percent suspension with 0.9-percent sodium chloride solution. The suspensions were cleared by centrifugation at high speed for 20 minutes in an ordinary centrifuge, and the supernatant fluid was administered orally to 7-to-14-day-old (I × C3H)F_1 hybrids. Each mouse received 0.2 cc. of the supernatant fluid. A total of 55 mice was used to test the 5 extracts; they lived to an average age of 20 months, and none developed a breast tumor.

Six other tumors from (C × C3H)F_1 mice were used to prepare a concentrated extract. These tumors occurred in mice 21 to 23 months old. The tumors were pooled, and a 25-percent extract was made by grinding with sand and diluting with 0.9-percent sodium chloride solution. The suspension was cleared by centrifugation for 30 minutes in an ordinary centrifuge. The cleared extract was spun in the ultracentrifuge at 62,000 × gravity for 1 hour and the supernatant fluid poured off. The pellet material was resuspended in 2 cc. of phosphate buffer solution (pH 7.0; M/15), giving a total volume of 2.4 cc. This gave a concentration 12.5 times that of the original extract. The concentrate was fed to 12 (I × C3H)F_1 hybrids and 11 strain C mice which were 2 to 3 days old, each receiving 0.1 cc. All lived to be 17 months old, and none developed a mammary tumor.

These results indicate that these tumorous (C × C3H)F_1 hybrids did not possess the milk influence.

DISCUSSION

The results confirm the occurrence of mammary tumors in hybrid F_1 mice obtained by mating strain C females to strain C3H males and indicate that a few such tumors may arise in (I × C3H)F_1 hybrids. The development of breast tumors in hybrids from low-mammary-tumor-strain females has been reported from several laboratories. Bagg and Jacksen (*12*) outcrossed C57 black females to CBA males as well as JK females to C57 black males and subjected the female offspring to a regime of intensive breeding. Of 32 (C57 black × CBA)F_1 hybrids, 20 developed breast tumors; and of 30 (JK × C57 black)F_1 hybrids, the first 5 to die each developed mammary cancer. The remaining 25 were alive when the paper was written. The authors state: "The indications are that the results from this cross will be similar * * *." Bittner (*13*) reported an incidence of 0.5 percent mammary tumors at an average age of 21.4 months in 568 breeding C57 black females; the average age at death was 20.8 months. When C57 black females were mated to A males, he procured 108 female hybrids which were bred and gave a tumor rate of 1.5 percent at an average age of 18 months. The average age at death was 21.1 months. Murray and Little (*14*) mated C57 black females to dba males and observed a tumor incidence of 6 percent in virgin hybrids; nonbreeding C57 black females did not develop mammary tumors. Strong (*15*) bred JK females to C3H males and obtained 24 F_1 female hybrids which were kept with males at all times. This procedure kept the females in an almost continuous state of lactation and pregnancy, and 17 developed mammary tumors.

The higher incidence among hybrids may be the result of a longer life span accompanying hybrid vigor, but this does not explain Bittner's results or those reported here. Breeding strain C females live to an average age of 19 months and have a tumor incidence of 1.4 percent.

It is generally agreed that at least three interrelated factors, genetic, hormonal, and milk influence, are involved in the production of mammary tumors in mice and that there is a quantitative balance between them. A deficiency of one factor may be overcome by an in-

crease in one or both of the others. The outcome of the investigation reported here raises the question whether a lack of a milk influence may be overcome by hormonal stimulation and inherited susceptibility. The evidence is suggestive that it may be but is not conclusive for the following reasons:

(1) Strain C milk may contain a small amount of influence, and the (C × C3H)F_1 hybrids may be more susceptible than the (I × C3H)F_1 animals. This possibility appears remote since it implies that the influence does not increase in amount in susceptible strain C mice which transmit it through successive generations. However, the observations of Bittner and of Miller and Pybus (*16*) suggest that the influence may even diminish in amount in successive generations of susceptible mice; and in view of their findings it is not inconceivable that strain C mice may transmit a small quantity of the influence from generation to generation.

(2) Strain C milk may contain an influence that is relatively weak. This assumption, or the assumption of a small amount of active influence in strain C milk, offers an explanation for the relatively high average age for tumor appearance in the hybrids, as well as for the unsuccessful attempts to detect the influence in the tumors. The high incidence of breast tumors in many generations of strain C mice following exposure to strain C3H milk could be due to the supplanting of a weak influence by a more active one.

(3) Also, an influence in strain C milk may differ from the known cancer-inciting influence in the milk of high-tumor strains. Law (*17–19*) found that the milk of certain mice contained an influence which promoted the growth of some transplantable tumors in normally refractory mice and obtained evidence that this influence differs from the mammary-tumor-inciting influence.

(4) The influence may have been produced in the (C × C3H)F_1 hybrids but in amounts too small to be detected in their tumors. Bittner (*20*) records the sudden appearance of the influence in a strain A female after seven generations of tumor-free mice. Fekete and Little (*21*) transferred fertilized ova from high-tumor-strain dba into the uteri of strain C57 black which nursed the young after birth. Thus, the dba mice did not have an opportunity to ingest dba milk. None of the mice born from the transferred ova developed mammary tumor, but there was an incidence of 11.7 percent in three generations of their descendants.

Hormonal stimulation may be of considerable importance in the production of breast tumors in hybrid mice. The animals in experiments reported by Bagg and Jacksen and by Strong, as well as those included here, were all subjected to intensive hormonal stimulation and showed a high incidence of tumors. If mammary tumors arise in hybrid mice in the absence of or with subthreshold amounts of the influence, the effect of hormonal stimulation could be expected to play an important part in their development.

Although the milk influence is of prime importance in the occurrence of mammary tumors in inbred strains, there is no reason to assume that all such tumors in mice are caused by its activity. The demonstration of the milk agent was dependent upon the use of inbred strains, and the milk of all high-mammary-tumor strains tested thus far contain the influence. Thus, the role of the milk influence in inbred strains is well established, but its importance in other mice has not received much attention. If the inbred mouse is responsible for the establishment or concentration of the milk influence, then the hybrid mouse should be a better test animal to ascertain whether mammary tumors occur in a considerable number of mice in the absence of the influence.

SUMMARY

Nineteen strain C female mice were bred to strain C3H males. Of 75 (C × C3H)F_1 females born to these matings, 39, or 52 percent, developed typical mammary tumors. Of 36 (I × C3H)F_1 females suckled by the

strain C females, only 1 developed a typical mammary tumor.

Extracts prepared from 5 tumors arising in $(C \times C3H)F_1$ hybrids were fed to 55 young $(I \times C3H)F_1$ mice, and none developed a tumor. An extract of 6 other tumors occurring in $(C \times C3H)F_1$ mice was concentrated 12.5 times by means of the ultracentrifuge and was fed to 12 young $(I \times C3H)F_1$ hybrids and 11 young C mice, and none developed a tumor.

The results show that mammary tumors arose in hybrid mice derived from strain C females and strain C3H males and suggest but do not prove that a milk influence was not involved in the occurrence of the tumors.

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SUSCEPTIBILITY OF YOUNG AND OF ADULT MICE TO THE MAMMARY-TUMOR AGENT

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Studies on the properties of the mammary-tumor agent of mice showed that it is widely distributed throughout the bodies of mice belonging to high-mammary-tumor strains (*1–3*) including their mammary tumors (*4, 5*). Subcutaneous or oral administration of the agent produced tumors in many young but in few adult mice (*3, 6*); older mice were apparently more resistant to the agent. The experiments reported at this time are a continuation of these studies and deal with the susceptibility of young and of adult animals to the agent.

Since young mice respond to the agent introduced orally, subcutaneously, or intraperitoneally, attempts were made to infect them by the growth of mammary-tumor cells within their tissues. The occurrence of tumors in only a few adults following administration of the agent suggested efforts to determine whether most are immune to infection or whether they are infected but do not develop tumor.

MATERIALS AND METHODS

Mice of strains C3H and I and their hybrids were used. All were born and raised in this laboratory. Female C3H mice develop spontaneous mammary tumors and contain the mammary-tumor agent; whereas strain I mice develop few such tumors, and their milk does not contain the agent. Their (I × C3H)F_1 hybrids are susceptible to the milk agent but do not obtain it from their mothers. Hence, they are satisfactory animals (*3*) for testing for the presence of the agent. All mice were kept under similar environmental conditions and were maintained on an unlimited supply of Purina dog chow pellets and tap water.

Tumor extracts were prepared by grinding freshly excised spontaneous mammary tumors with sand and diluting with 0.9-percent sodium chloride solution to give a final concentration of 20 percent by volume of tumor tissue. The suspensions were cleared by centrifugation at top speed for 20 minutes in an ordinary centrifuge, and the supernatant fluid was used as a source of the agent.

Tumors were implanted in the skin of young mice by the following technique: Freshly excised spontaneous mammary tumors arising in mice known to possess the mammary-tumor agent were passed through a fine mincing machine. Inoculations were made by using a 1-cc. tuberculin syringe and a 21-gage needle. The needle was inserted just beneath the skin so that its bevel was plainly visible, and a small amount of the minced tumor was expelled from the syringe. The resultant tumors were removed with ordinary surgical procedure while the animals were under nembutal anesthesia.

ATTEMPTS TO INFECT YOUNG MICE BY TUMOR INOCULATION

Three experiments were performed: In one, a mammary tumor from a (I × C3H)F_1 hybrid, and in the others mammary tumors from C3H females were used as inoculums. Young (I × C3H)F_1 hybrids from 2 to 10 days of age were inoculated, and the implanted tumor cells grew in every mouse. The tumors were removed when their average dimensions were 15 x 6 mm. When the mice were 2 to 3 months old, they were mated and permitted to bear one litter which was killed within 24 hours after birth.

The results are summarized in table 1. The third column of the table shows the number of days elapsing between inoculation and removal of the tumor. Of 23 mice, only 1 developed a mammary tumor. The results indicate that the growth of the first transplant generation of spontaneous mammary tumors did not infect the mice with sufficient amounts of the

TABLE 1.—*Response of (I×C3H)F₁ hybrids that grew the first transplant generation of spontaneous mammary tumors, to the development of mammary tumors in later life*

Number of mice	Age when inoculated	Time exposed to tumor cells	Number developing mammary tumor	Age when tumor occurred	Number dying without mammary tumor	Average age at death without tumor
	Days	*Days*		*Months*		*Months*
7	6	26	0	.....	7	18.4
3	2	28	0	.....	3	16.6
5	3	28	1	10.0	4	18.2
2	10	28	0	.....	2	19.5
2	3	40	0	.....	2	20.0
2	10	40	0	.....	2	17.5
1	10	43	0	.....	1	14.0
1	10	53	0	.....	1	21.0

agent to produce tumors in later life. The tumor arising in 1 mouse was not tested for the presence of the tumor agent.

ATTEMPTS TO DETECT INFECTION IN ADULT MICE

Two experiments were performed to detect infection in adult mice. The first was started by mating 9 strain I females to strain C3H males. Twenty-five female progeny were born during September and October 1942. These were mated when 3 months old, and each bore one litter during December 1942 or the first part of January 1943. The litters were killed within 24 hours after birth. On January 22, 1943, each mouse received an injection of 0.5 cc. of tumor extract prepared from a spontaneous mammary tumor of a strain C3H female. Twelve were given the material subcutaneously, and thirteen intraperitoneally. The tumor extract was proved to contain the agent by feeding 0.2 cc. of it to each of twelve 10-day-old strain C mice, of which eight developed mammary tumor at an average age of 10 months.

Eight days following injection, the 25 (I × C3H)F_1 hybrids were mated to strain C3H males, and each bore from 1 to 3 litters. The offspring of this mating were (IC3H × C3H)BC animals and were susceptible to the agent since they were derived by backcrossing to the susceptible C3H strain. A total of 115 (IC3H × C3H)BC females was obtained. Each was bred when 2 months old and gave birth to 1 litter, which was killed soon after birth. The (IC3H × C3H) BC mice and their (I × C3H)F_1 mothers were observed for the appearance of mammary tumor.

The results are presented in table 2. Two of the (I × C3H) F_1 mothers developed mammary tumor; the others lived to an average age of 25 months. In a total of 40 (IC3H × C3H) BC mice born to 7 of the F_1 mothers, 25 developed mammary tumors; whereas in a total of 75 offspring raised by 14 of the F_1 mothers, none developed a breast tumor.

Tumors found in the (IC3H × C3H) BC animals were not tested for the presence of the mammary-tumor agent, and the question arises whether it was involved in the production of these growths. Recent findings (*8*) suggest that the agent was not responsible for breast tumors in F_1 hybrids derived from strain C females and C3H males. The (C × C3H)F_1 hybrids experienced intensive hormonal stimulation since they bore 3 litters in rapid succession whereas the (IC3H × C3H)BC mice of the present investigation had but 1 litter. When all mice of both groups were 1 year old, the (C × C3H)F_1 hybrids showed a tumor incidence of only 3 percent, and the (IC3H × C3H)BC an incidence of 22 percent. If it is assumed that the agent was not involved in the occurrence of tumors in the (IC3H × C3H)BC animals, then they must have been much more susceptible to hormonal stimulation than were the (C × C3H)F_1 hybrids. Further, tumors appeared in the litters born to 17 of 19 (C × C3H)F_1 hybrids and in only those litters born to 7 of 21 (I × C3H)F_1 hybrids. Any genetic influence would have to be limited to a third of the litters born to the (I × C3H)F_1 hybrids.

The fact that all tumor-bearing (IC3H × C3H)BC mice were confined to seven mothers suggests that their mothers were infected by the agent and transmitted it in their milk. Additional evidence that the milk of the seven (I × C3H)F_1 mothers contained

TABLE 2.—*Occurrence of mammary tumors in (I × C3H) F_1 adult hybrid mice following injection with mammary tumor extract, and in their progeny*

Mouse No.	Route of injection [1]	Age when mammary tumor occurred	Age at death without mammary tumor	Number of females weaned	Number developing mammary tumor	Average tumor age	Number dying without tumor	Average age at death without tumor
		Months	*Months*			*Months*		*Months*
1	SC		26.5	6	1	12.0	5	19.4
2	SC		26.5	5	0		5	19.5
3	IP		26.5	0				
4	IP		26.5	5	0		5	15.6
5	SC		26.5	3	0		3	20.0
6	IP	23.5		11	10	10.6	1	16.5
7	SC		25.5	4	3	10.0	1	21.0
8	IP		25.5	5	0		5	20.0
9	SC		17.5	7	0		7	19.0
10	SC		26.0	5	4	11.9	1	14.5
11	IP		26.0	9	0		9	20.5
12	SC		24.5	2	0		2	19.0
13	IP		26.0	4	0		4	21.0
14	IP		26.0	13	0		13	18.9
15	SC	20.0		0				
16	IP		25.5	4	3	11.0	1	20.0
17	SC		25.5	9	0		9	20.3
18	IP		24.5	7	0		7	19.4
19	IP		25.5	4	1	9.0	3	19.0
20	SC		25.5	6	3	14.2	3	19.3
21	IP		25.5	0				
22	IP		25.5	2	0		2	18.3
23	IP		25.5	1	0		1	20.0
24	SC		25.5	0				
25	SC		25.5	3	0		3	20.0

[1] SC, subcutaneous; IP, intraperitoneal.

the agent is supplied by the tumor history of their litters. This evidence is given herein.

Female No. 6, which developed a tumor, raised 11 progeny, of which 10 developed tumor. Her first litter died; the second contained 5 females of which 4 became tumorous; the third contained 6 females, and all had tumor. F_1 mouse No. 15, which developed a tumor, gave birth to 2 litters. One died, and the other consisted of males only. Mice Nos. 7, 16, and 19 each bore 2 litters, but their first died. Results in their second litters are given in table 2. The others each bore 3 litters. Mouse No. 1 lost her first litter; her tumorous offspring was in her second litter, which contained 5 females. Mouse No. 10 raised her first 2 litters; the first contained 1 female which developed a tumor, and the second consisted of 4 females of which 3 had tumors. Mouse No. 20 lost her first litter; the second contained 5 females, and 2 became tumorous; the third contained 1 female which developed a tumor. With the exception of 2 tumorous mice born to mice Nos. 1 and 9, the foregoing data are consistent with what one would expect if the milk of the hybrid mothers contained the agent. It is known (*6*, *9*) that female mice can transmit the agent in their milk even when they do not develop breast tumors.

The results of this investigation confirm the earlier work (*3*) concerning the resistance of adult mice to the development of tumors following administration of the mammary-tumor agent and suggest that susceptible adults may be infected and transmit the agent in their milk although they do not develop tumor.

The second investigation was similar to the first except that strain I females were used instead of (I × C3H)F_1 females. Strain I mice are relatively resistant to the development of mammary tumors, and their milk does not contain the agent. Thirteen strain I females born during June 1942 had each borne 1 litter. On October 28, 1941, each received 0.5 cc. of tumor extract prepared from a spontaneous mammary tumor from a female C3H mouse. Six received the extract sub-

cutaneously, and seven intraperitoneally. They were mated to strain C3H males 7 days after the time of injection. Thus, their offspring were susceptible (I × C3H)F_1 hybrids. Each strain I female gave birth to 1 or 3 litters, and 69 (I × C3H)F_1 females were obtained. Each of these progeny gave birth to 1 litter, which was killed soon after birth.

The strain I females lived to an average age of 12 months, and none developed a tumor. Fifty-six of their (I × C3H)F_1 offspring lived to an average age of 21.7 months and failed to develop a tumor.

The remaining 13 hybrids were fed mammary-tumor extract to test their resistance to the agent. It was thought that if the injection of the agent elicited neutralizing antibodies in the strain I mothers, their offspring might be resistant to infection in early life. The 13 test mice represented 8 litters which also contained 7 other females that remained tumor-free. When the test mice were 7 to 14 days of age, each was fed 0.2 cc. of strain C3H mammary-tumor extract. Nineteen other (I × C3H)F_1 hybrids of the same age, whose mothers had not been injected, served as controls, and 15 developed tumor at an average age of 10 months. The 13 test mice lived to an average age of 20 months and were free of tumor. This exploratory study suggests that the injection of tumor extract elicited antibodies in the strain I females and that immunity was transferred to their offspring. This problem is receiving further consideration.

In contrast with the results of the first experiment in which progeny of (I × C3H)F_1 females developed tumors, the offspring of the I females in this experiment remained tumor-free. Apparently the agent did not infect the mammary-gland tissues of resistant strain I mice. Indeed, there is evidence that the adult I females developed neutralizing antibodies following injection of the agent.

The outcome of both investigations reveals that a subcutaneous or intraperitoneal injection of mammary-tumor extracts known to contain the mouse mammary-tumor agent into susceptible or resistant adult mice produced few tumors in the susceptible, and none in the resistant animals.

SUMMARY

Young susceptible (I × C3H)F_1 hybrid mice received cutaneous implants of tissue from spontaneous mammary tumors occurring in mice which were infected with the mouse mammary-tumor agent. The transplants grew in all the mice and remained in them from 26 to 53 days when they were removed. Of 23 mice that grew the implanted tumors, 1 developed a spontaneous mammary tumor in later life. Apparently cutaneous growth of tumor cells is not an effective means of infecting these young susceptible mice with the mammary-tumor agent.

Adult female mice of resistant strain I and susceptible (I × C3H)F_1 hybrid derivation were given a single subcutaneous or intraperitoneal injection of 0.5 cc. of mammary-tumor extract. They were then mated to appropriate males, and the female offspring were kept. Of 25 (I × C3H)F_1 females, 2 developed mammary tumor. Twenty-one nursed female progeny, and 7 raised 40 mice, of which 25 developed mammary tumors, whereas 14 raised 75 mice, none of which became tumorous. None of 13 strain I females developed a tumor, and none of their 69 (I × C3H)F_1 progeny did so.

The results indicate that some of the susceptible (I × C3H)F_1 adults became infected with the mammary-tumor agent and transmitted it in their milk, whereas none of the resistant strain I adults became infected.

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ESTRUS IN VIRGIN STRAIN C3H (HIGH-TUMOR) AND VIRGIN STRAIN A (LOW-TUMOR) MICE AND IN THE RECIPROCAL (A × C3H) F_1 HYBRIDS

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INTRODUCTION

The production of mammary tumors in mice is dependent upon at least three interrelated factors, namely, the genetic susceptibility, the hormonal stimulation, and the milk agent. Although both strain C3H and strain A possess the milk agent, they differ in that whereas the incidence of mammary tumors is high in both breeding and virgin strain C3H females, it is high only in breeding strain A females. From a study of these two strains and their reciprocal hybrids, Heston and Andervont (*1*) demonstrated that the difference in incidence in the virgins of the two strains was owing fundamentally to a difference in the genic complex of the two strains. A similar experiment was reported by Bittner et al. (*2*). When strain A females were mated with strain C3H males, it was found that the resultant (A × C3H)F_1 hybrids had a high tumor incidence which was comparable with that shown by strain C3H females. The introduction of the C3H chromatin of the father was sufficient to raise the tumor incidence from the low percentage of the A females to a high percentage comparable with that of the C3H females. The reciprocal (C3H × A)F_1 hybrids likewise had a high tumor incidence.

It was thus apparent that the fundamental difference between the two strains was one of genetic factors. The question arose whether these genetic factors were causing a variation in amount of hormonal stimulation or whether the variation was a difference in genetic susceptibility to the hormonal stimulation. Certain evidence pointed to a difference in hormonal stimulation. This was shown by experiments in which virgin strain A females injected with estrogens developed tumors (Gardner (*3*) and Suntzeff et al. (*4*, *5*)). Breeding strain A females develop tumors, and Jones (*6*) showed that the effect is additive, the percentage of tumors increasing in direct proportion to the number of litters produced. However, it was not demonstrated conclusively that the hormonal stimulation in strain A differed from that in strain C3H.

It therefore seemed desirable to secure further evidence whether or not there was a difference in hormonal stimulation because the demonstration of such a difference would indicate that the genetic influence was manifested through the hormonal mechanism. In the present investigation, the problem has been attacked through a study of the estrus cycle of these two strains and their reciprocal hybrids.

MATERIALS AND METHODS

Strain C3H and strain A mice of the sublines maintained at the National Cancer Institute were used. Daily vaginal smears were taken on 10 virgin strain C3H mice, 10 virgin A mice, and on 10 virgins each of the 2 reciprocal hybrid groups, (C3H × A)F_1 and (A × C3H)F_1. Beginning at 3 weeks of age, daily observations were made to determine the exact age at which the vaginae opened. The smears were first taken on the day that the vaginae opened and were continued until the animals were 150 days of age. The pipette method (*7*) was used, and the smears were stained with methylene blue.

The animals were housed in wooden cages with but four animals to the cage since Andervont (*8*) suggested that crowding or contact interferes with hormonal stimulation. He took a series of vaginal smears on segregated (one to a cage) and nonsegregated (eight to a cage) virgin C3H litter mates and found that the estrus cycles occurred earlier, more frequently, and were of

greater duration in the segregated animals than in the nonsegregated ones.

The animals were fed Purina dog chow and were constantly supplied with tap water.

RESULTS AND DISCUSSION

The results obtained, summarized briefly, are presented in table 1. It is evident from the table that in the groups studied there is no significant difference in the average length of the estrus cycle. A regular cycle of 4 to 5 days' duration in the different groups is indicated. These results agree with those obtained by Allen (*9*) and by Burns, Moskop, Suntzeff, and Loeb (*10*). Allen stated that the duration of the estrus cycle in mice is from 4 to 6 days. Vaginal smears which he took on several different strains of mice showed the following variation in the length of the estrus cycle; for brown mice, 6 days; for black mice, 4 days; for white mice (brown minus the color factor), 4 days; for yellow mice, 5 days; and for gray mice, 5 days. Burns et al. (*6*) in their study of the relationship of the incidence of mammary cancer and the sexual cycle in different strains of mice concluded that there was no connection between the different characteristics of the sexual cycle and the frequency of mammary cancer in the 10 different strains of mice studied. In their study they determined:

(a) The duration of estrus (period of keratinization in vaginal epithelium) in individual mice; (b) the average duration of estrus in the various strains; (c) the total number of days of keratinization during a given period in the individuals of each strain and the averages for the various strains; (d) the average number of estrous cycles in each strain; (e) the normality or regularity of the estrous cycles in the strains and the individual mice comprising them.

It was thought that perhaps if the difference in incidence was due to a difference in hormonal stimulation, it might manifest itself in the length of the estrus cycle in the two strains and their hybrids. Thus one might have expected shorter and consequently more frequent cycles in mice showing a higher tumor incidence. However, it is clear that no such relationship exists in the present experiments.

TABLE 1.—*Estrus in the various groups of virgin females*

Type of females	Opening of vagina (average age)	Length of estrus cycle (average)
	Days	*Days*
A	45.6	4.52
C3H	35.0	4.67
$(A \times C3H)F_1$	31.7	4.59
$(C3H \times A)F_1$	36.6	4.49

The striking difference between the groups was the variation in average age at which the vaginae opened. It was found that the vaginae opened at a later age in the A than in the C3H females or in either hybrid group. Statistical analysis of the data indicates a significant difference in the average age of the strain A as compared with that of the strain C3H and the two hybrid groups. In a comparison of strain A with strain C3H, $t=6.22$; with $(A \times C3H)F_1$, $t=9.92$, and with $(C3H \times A)F_1$, $t=3.82$; $P<0.01$ in all cases.[1] In a comparison of the two hybrid groups $(C3H \times A)F_1$ and $(A \times C3H)F_1$, $t=2.13$, and P is between 0.02 and 0.05, which is bordering on significance. Correlation can be noted between the age at which the vaginae opened and the percentage incidence of mammary tumors in the different groups of animals. Heston and Andervont (*1*) published the following incidences: A, 0; C3H, 93; $(A \times C3H)F_1$, 91; and $(C3H \times A)F_1$, 83 percent. Bittner et al. (*2*) published similar data on cancer incidence for virgins 9 months or older, which are as follows: A, 0; Z or C3H, 43; AZF_1, 45; and ZAF_1, 20 percent. They suggested that the difference in the incidence of mammary-gland tumors in virgin A and virgin C3H mice is the result of characteristic differences in hormonal metabolism. These differences may result from—

(a) the amount of estrogen produced or available for the stimulation of the mammary glands, and/or (b) the sensitivity of the mammary glands to estrogenic hormones.

[1] These probabilities were calculated with the use of the t statistic of Fisher (*11*).

The present experiments indicate a difference in hormonal stimulation since the opening of the vaginae, which is affected by hormonal stimulation,occurs earlier in the types of mice showing a higher tumor incidence. These findings contribute evidence that the difference between the strain C3H virgin females with a high tumor incidence and the strain A virgin females with a low tumor incidence, which has been demonstrated to be a genetic difference, is manifested in part at least through the hormonal mechanism. It should be emphasized, however, that the demonstrated difference is not great, probably representing but two estrus cycles, and thus a variation in genetic susceptibility to the hormonal stimulation may still be a more influential difference unless it can be proved that these few early cycles are the most important ones.

SUMMARY

The estrus cycles of inbred strain C3H and strain A mice and their reciprocal F_1 hybrids were followed for a period of 150 days. The presence of a regular cycle of 4 to 5 days' duration was indicated in the four groups studied.

There was considerable variation in the age at which the vaginae opened in the different groups, the vaginae opening significantly later in strain A as compared with strain C3H and the two hybrid groups $(A \times C3H)F_1$ and $(C3H \times A)F_1$.

In comparing the age at which the vaginae opened in the different groups with the mammary-tumor incidence, it was found that within these groups those in which the vaginae opened at an earlier age had a higher tumor incidence.

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POLAROGRAPHIC RESPONSE OF DEPROTEINATED SERUM OF INDIVIDUAL RABBITS BEFORE AND AFTER IMPLANTATION WITH THE BROWN-PEARCE CARCINOMA

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INTRODUCTION

The blood or serum proteose[1] has been found to be increased in animals bearing cancers, as well as in various other pathologic states (*1*). Considerable discussion is extant as to the possible usefulness of an estimation of the quantity of this material present. The study reported herein was undertaken to answer certain of the questions posed by the investigations so far relative to (1) the constancy from day to day, of the proteose content of the serum, estimated polarographically, in a single animal, (2) the comparison of such values with those from other animals to establish the normal range, (3) the study and comparison of the polarographically determined wave height before and after implantation with a tumor, (4) the correlation of the wave height after the tumor became well established with its size and metastatic extension, (5) the comparison of the wave height of the serum filtrates from those animals that were planted with the tumor but in which it failed to grow, with the normal and cancer-bearing ones, (6) the initial stage at which the tumor could be detected inasmuch as any change might have maximum diagnostic usefulness if it accompanied a small or beginning growth,[2] and (7) the differentiation of the polarographic response given by cancer serum from other pathologic states.

The requirements listed imposed certain limits with respect to material available. The need for several blood withdrawals in considerable quantity militated against the use of the mouse and rat as experimental subjects. Rabbits were chosen because they were available in considerable number incidental to other work, and the Brown-Pearce carcinoma was selected for implantation because it was being carried routinely at this Institute. Winzler and Burk had shown with five rabbit-blood samples that the tumor gave polarographic waves well above the normal average for other rabbits, although the upper range of the latter attained the lower range of the former, when the oxalated whole blood proteins were removed by precipitation with 0.2 M sulfosalicylic acid (salicyl-4-sulfonic acid), hereafter referred to as SSA. Another point favoring the use of this tumor was the well-established finding that it metastasizes extensively, a characteristic deemed desirable for the reason noted under (4). Finally, the normal proteose content of rabbits' blood measured in this way was in the lower range for animals so far investigated. It was thought that the lower the normal quantity of proteose, the easier it would be to detect any change from this. The relative increase in the proteose content of serum of rabbits bearing a well-developed tumor was of the same order as that for the Jensen-tumor-bearing rats even though the normal rat-serum proteose estimated in this way was three to four times as great in amount as that of the rabbit. Perhaps some reasons against the use of the Brown-Pearce tumor were that it is transplanted and possibly was induced (*3*, *4*) and that it frequently kills the host in from 30 to 60 days.

Obviously any study such as this in which serial blood sampling was made must suffer from the defect that necropsy could not be done immediately after each sample was withdrawn.

[1] See reference (*1*) for a discussion of the entity "proteose" and the literature pertinent to its behavior in clinical and experimental cancer. As used herein the term refers to the nondialyzable material left in solution following removal of the serum proteins with sulfosalicylic acid.

[2] While this paper was being prepared, there came to hand a report by Merten and Bernot (*2*) on their work with the Brown-Pearce tumor, among other things. They found no increase in the polarographically active groups of the blood until general systemic effects attributable to the tumor were established. It has not been possible to secure more than an abstract of this article.

MATERIALS AND METHODS

The rabbit stock was the usual assortment of adult males of diverse previous history, housed in individual cages, and kept in quarantine for some weeks after arrival at the laboratory. The attic room where they subsisted showed a temperature range from 18° to 30° C. in the course of the investigation, which covered about a year. There was a considerable incidence of mild nasal discharge, referred to herein as sniffles, and a few animals were lost because of pneumonia. Whether chilling with subsequent return to normal room temperature has any effect on the proteose content of the blood is not known.[3] The animals were maintained on Ralston Purina rabbit chow and whole grain oats and had free access to bottled tap water. They were studied in lots of 6, mainly because this was a convenient number for making the tumor transfer. Owing to various circumstances, it was seldom possible with an initial set of 6 rabbits to secure complete data for a month before planting and through to necropsy. Because of these circumstances, about 50 had records complete enough to be included in the final tabulations.

Blood sampling was usually dropwise from the marginal ear vein after splitting it longitudinally with a razor blade. An effort was made to secure samples at 4-day intervals, but this was not always possible. About 2 to 7 cc. blood was drawn at any one time, allowed to clot at room temperature for an hour, and then preserved at 4° C. overnight. Of this serum [4] 0.2 cc. was measured into 3.8 cc. of 0.245 M SSA at room temperature. The solution was poured onto a 9-cm. Whatman No. 5 filter paper after 10 minutes. Filtration was usually complete in another 15 minutes. One cubic centimeter of the filtrate was treated with 2 cc. of the reagent as previously described (*1*), and the polarographic response was determined. Analyses were run in duplicate unless otherwise noted. By duplicate is meant that two separate precipitations and filtrations of serum were made; not that two blood samples were taken at one time from one animal. When two estimations are recorded on one filtrate, they are referred to as aliquots to distinguish them.

As the work proceeded, some attention was given to the limits of precision of the method used. As can be inferred from what has been said concerning handling the animals in lots of 6, any one set of analyses involved a minimum of 12 estimations for the blood-serum filtrates. Along with each set, at least 3 recordings of the height of the reagent blank were made since it is the difference between this base line and the peak of the serum filtrate wave that is of interest. Perusal of the literature reveals that this is the reference standard universally used. This has been found to be not quite correct for rabbit serum. Ultrafiltrates of the serum prepared by using Paraloidin membranes of such porosity as to allow the passage of inulin of molecular weight 5,000 (*6*) but not protein gave a value slightly above the blank, i. e., 33.6 against 33.3.[5] Dialysis through No. 300 cellophane against an equal volume of Ringer's solution gave a similar result. Whether this is due to the fact that some of the proteose molecules are of such small size that they can pass such membranes or to changes induced by the dialysis is not known. For convenience, the simple reagent blank, 1.0 cc. of 0.233 SSA plus 2 cc. of the cobaltammine,[6] has been used throughout this study, but the reservation mentioned should be borne in mind. Within the limits of the method, this diffusible portion seemed to be constant for 1 animal and on the comparison before and after planting the tumor should cancel out.

[3] It is possible that such extraneous factors as light and heat may have some effect. Brown and Howard (*5*) found some effect produced on the growth and nutrition of caged normal rabbits by variation in illumination.

[4] This differs from the procedure of Winzler and Burk (*1*) in that they used oxalated whole blood.

[5] That this is likewise the case for normal rat's blood may be deduced from Winzler and Burk's table 3.

[6] It was found that of the commercially available preparations of the cobaltammine some contained as much as 20 percent impurity, hence all such preparations were recrystallized before use.

It was early apparent that there was some variation in consecutive estimations both of the blank and of the filtrates. Table 1 gives representative results under several conditions. It was possible to read the graph to 0.1 chart unit where the recorder range was 100 units, equivalent to approximately 1 ma. per unit, and this range covered a width of the paper of 250 mm. By using duplicate estimations, the results for the blank might be removed from the most probable value of the series by 0.2 unit. Any 2 values from the last column of the table could represent the usual experimental findings on a normal animal. This shows the results in practice of the over-all accumulation of errors ascribable to all the known factors, neglecting the possible 0.3 unit in absolute terms from the undialyzed blank. There exists then some chance that the analytical value for a particular serum might deviate from the most probable value by 0.4 unit. This deviation produces a relatively large uncertainty in experimental figures when the values are around 1 to 2.5 as in the normal rabbits. Table 2 is included largely by way of emphasizing this point. It lists the difference between the reagent blank of table 1 and the individual estimations on the serum shown in the table. As pointed out, it is this difference that must be the basis of inquiry into deviation from the normal when quantitative differences are sought. Particular attention is called to the first figure of the third column of the table which deviates from the mean by 75 percent. In the early days of this work when oxalated whole blood was used, an occasional filtrate would be opalescent as if protein were incompletely removed, and the proteose wave was higher than its duplicate. A third filtrate, made from the same blood, if clear, invariably agreed more closely with the lower value. The serum filtrates were always clear, water-white, but it would appear that an aberrant figure such as this must be due to some filters' having a few pores larger than most. As nearly as can be determined by inspection, such filtrates are the same as any other. They have not been encountered on the filtered blank estimations (column 2, table 1) but seem to occur about once in every 30 estimations on the serum filtrates.

TABLE 1.—*Reproducibility of polarographic wave heights*

Blank on reagents	Filtered blank	Aliquot samples from 1 filtrate [1]	Single precipitation, 10 aliquots filtered [1]	Replicate serum filtrates [1]
33.3	33.6	34.3	35.4	34.2
33.2	33.4	34.4	34.3	34.2
33.6	33.4	34.7	34.6	34.2
33.3	33.5	34.5	34.3	34.5
33.1	32.9	34.8	34.4	34.2
33.4	33.3	34.9	34.2	34.4
33.4	33.3	34.9	34.2	35.1
33.3	33.3	34.9	34.6	34.4
33.1	33.4	34.4	34.7	34.1
33.2	33.2	34.6	34.3	34.8
33.3 ±.11	33.3 ±.14	34.6 ±.20	34.5 ±.24	34.4 ±.24

[1] Serum used from a normal rabbit's blood drawn from the jugular vein under nembutal-ether anesthesia.

TABLE 2.—*Difference of blank mean from individual estimations of each serum filtrate series of table 1*

Aliquot samples from 1 filtrate	Deviation from series mean	Ten aliquots filtered	Deviation from mean	Replicate filtrates	Deviation from mean
1.0	0.34	2.1	0.9	0.9	0.2
1.1	.24	1.0	.2	.8	.3
1.4	.06	1.3	.1	.9	.2
1.2	.14	1.0	.2	1.2	.1
1.5	.16	1.1	.1	.9	.2
1.6	.26	.9	.3	1.1	.0
1.6	.26	.9	.3	1.8	.7
1.6	.26	1.3	.1	1.1	.0
1.1	.24	1.4	.2	.8	.3
1.3	.04	1.0	.2	1.5	.4
1.34	.2	1.2	.26	1.1	.22

RESULTS

The initial experiments on the rabbits included one blood sample to establish the normal value just before planting with the tumor tissue and another after the tumor developed. Such a set is shown in table 3 from some of the animals Nos. 606 to 615. The final blood samples from these animals were obtained under nembutal-ether anesthesia. This anesthetic mixture had no effect on the proteose wave. An experiment on its use as an anesthetic is summarized in table 4, chiefly because in addi-

tion to showing that there is no effect produced by nembutal-ether used in this way, it records the variation in the serum-proteose value during 1 day and a comparison of consecutively taken serum samples. From what has been said previously and from the data of this table, it appears that variations within the range shown here are no more than might arise from the errors of the method. Prolonged ether anesthesia has been shown to produce serum concentration as much as 10 percent (7). Presumably this increase in the percent of protein would be accompanied by a similar increase in proteose. An increase of this order is within the reproducibility range of the method and with few animals would escape detection. Ether was used for an interval of no more than 10 minutes so that in any event there would be a minimum effect from it. Attention is called to the results for rabbit No. 695. This animal was planted with a portion of the same inoculum as the others of the 694–699 group but failed to develop any tumor tissue, and its proteose wave is lower than that of its serial mate No. 696. Of the approximately 100 animals for which some data were recorded, 5 failed to develop tumors following implantation.

TABLE 3.—*Effect of the Brown-Pearce tumor on the proteose of rabbit's serum*

Rabbit No.	Day of bleeding [1]	Wave height in chart units	Necropsy record
606	0	2.0	Small tumor (1 gm.) in left testicle; nothing elsewhere.
	43	3.5	
610	0	2.5	Two medium testicular tumors (8 gm.); extensive intraperitoneal and omental metastases, several in lungs, liver, and diaphragm.
	20	4.5	
613	0	1.5	One small testicular tumor; one small metastasis on node in peritoneal cavity.
	20	2.5	
614	0	1.5	Two medium testicular tumors; very extensive metastasis in omentum, liver, lungs, and eyes.
	20	4.5	
615	0	1.5	Two medium testicular tumors; few metastases in liver, lungs, and omental nodes.
	20	2.5	

[1] First blood from ear vein on 0 day just before inoculation, second was fatal bleeding from heart under nembutal-ether anesthesia.

TABLE 4.—*Proteose content of serum from blood taken at different times during 1 day*

Rabbit No.	Time from first blood sample	Proteose wave height in chart units	Remarks and necropsy record
	Hours		
695	0	2.3	Planted with Brown-Pearce tumor 63 days before date of experiment. No tumor developed.
	2	2.85	
	4	3.2	
	4.1	2.65	
	6	2.75	
	[1] 6.5	2.55	
696	0	4.5	Planted with Brown-Pearce tumor as above. Two medium (9 gm.) testicular tumors; 2 large (20 gm.) metastases to spermatic cord, few to abdominal lymph nodes and to both eyes.
	2	4.6	
	4	4.8	
	4.1	4.5	
	6	4.0	
	[1] 6.5	4.5	
700	0	3.9	Planted with Brown-Pearce tumor 41 days before date of experiment. Two large (24 gm.) testicular tumors in abdomen (retained); small metastasis on one spermatic cord, several to lymph nodes in abdomen and to both kidneys.
	2	4.1	
	4	4.1	
	4.1	4.3	
	6	4.1	
	[1] 6.5	4.0	
702	0	5.6	Planted same as No. 700. One medium testicular tumor, medium metastasis to spermatic cord on that side. Coccidiosis egg sacs in abdomen.
	2	6.1	
	4	5.1	
	4.1	5.7	
	6	4.7	
	[1] 6.5	5.3	

[1] Killed under nembutal-ether anesthesia at this time.

Table 5 summarizes another portion of the data. It shows simply that with well-developed tumors there is, in general, an increase in the wave height and that it takes some time for this increase to become striking. This extends to the rabbit what Winzler and Burk found for the rat. The mean preinoculation value for the wave height of this single set was 20 percent lower than that for the majority of the rabbits for which four to five serum samples were analyzed before inoculation with the tumor. It was thought that there might be some increase in the wave height resulting from the repeated bleedings, inasmuch as hemorrhage has been shown to increase polypeptidemia (*8*), and Winzler and Burk (*1*) presented evidence that polypeptidemia and proteosemia were the same. However, no increase in the proteose wave height could be demonstrated as due to the relatively small blood withdrawals of our experimental conditions. Some of the data bearing on this point are listed in table 6. This experiment was per-

formed chiefly to see whether there would be any effect produced by inoculation of normal testicular material into the testes of normal rabbits in exactly the same fashion as for the regular testicular-tumor inoculations. Six rabbits were bled at five different times during the 20 days preceding and in the 33 days following inoculation. The table shows that there was no increase in the wave height; but rather, so far as there seemed to be any trend, it was downward. Added to this table are data for rabbit No. N–10, not inoculated but bled daily for 54 consecutive days; the data include only those samples falling on the days when the others were examined but also include the maximum and minimum found for this animal during this interval. They likewise support the conclusion that in the absence of infection or a tumor the wave height is relatively constant for an individual animal.

TABLE 5.—*Polarographic response of rabbit's serum before implantation of the Brown-Pearce tumor and during the growth of the implant* [1]

Rabbit No.	Time of sample [2]	Wave height in chart units	Necropsy record
	Days		
664	0	2.5	Killed by intravenous injection of air at 19 days. Two small testicular tumors; 2 small metastases on spermatic cord, 2 on diaphragm, and 5 on liver.
	8	2.1	
	11	4.0	
	18	4.5	
665	0	2.0	Killed as above. Two medium testicular tumors (8 gms.); 2 moderately large (4 gm. each) metastases to spermatic cord, 1 small to mesenteric lymph node, 4 in liver, and 2 in lungs.
	8	2.1	
	11	5.5	
	18	5.0	
666	0	1.5	Killed as above. One small testicular tumor; small (1 gm.) metastasis on spermatic cord on that side.
	8	1.4	
	11	3.5	
	18	5.0	
667	0	2.0	Killed as above. Very small amount of tumor tissue in each testis; 1 small metastasis to cord.
	8	1.8	
	11	3.0	
	18	5.0	
668	0	1.5	Killed by decapitation on forty-sixth day. Two medium testicular tumors (18 gm.); extensive coccidiosis with egg sacs in omentum.
	8	1.4	
	11	3.0	
	18	4.0	
	32	3.5	
	37	4.0	
	40	3.0	
	43	3.5	
669	0	1.5	Killed by air as above. Two very large (24 gm. each) testicular tumors; no metastases.
	8	1.4	
	11	3.0	
	18	4.0	

[1] Growth of implants just detectable on the eighth day; tumors easily palpable on the eleventh.
[2] Blood sample on 0 day taken just before inoculation.

TABLE 6.—*Proteose content of serum of normal rabbits before and after implantation with normal rabbit testicular tissue*

Time of sample (In days)	Wave heights in chart units for rabbit No.—						
	N-1	N-2	N-3	N-4	N-5	N-6	N-10
−20	2.55	3.55	2.65	3.5	2.8	2.55	2.05
−16	2.95	2.45	1.9	3.2	2.95	2.4	2.15
−8	2.7	2.4	2.65	3.55	2.8	2.1	2.4
−3	2.15	2.2	2.85	2.7	1.9	2.2	2.1
0	2.3	2.0	2.3	2.5	1.35	1.65	1.75
+5	2.05	2.5	2.95	2.5	2.4	2.9	2.0
+8	2.7	2.4	1.4	2.95	2.25	1.4	1.5
+16	1.75	1.7	[1] 1.7	[1] 1.5	[1] 1.8	[1] 2.0	3.0
+19	1.7	1.5	2.25	2.1	1.2	[1] 1.1	2.1
+33	2.05	1.5	1.25	2.7	1.65	1.65	2.5

[1] Single analysis only on these.

TABLE 7.—*Representative proteose values for rabbit's serum before and after implantation with the Brown-Pearce tumor when more than 1 pre-inoculation sample was taken*

Rabbit No.	Time of sample	Wave height in chart units	Necropsy record and remarks
	Days		
661	−5	0.9	Large amount of tumor tissue at necropsy. Animal moribund on +22 day and died 4 hours after this sample was taken.
	−2	.6	
	0	1.4	
	+7	1.3	
	+10	2.0	
	+15	6.5	
	+22	3.3	
662	−5	1.5	Large amount of tumor tissue at necropsy. Moribund on + 22 day and died 6 hours after bleeding.
	−2	1.1	
	0	1.3	
	+7	1.4	
	+10	2.4	
	+15	4.5	
	+22	1.9	
671	−19	2.4	Large amount of tumor tissue at necropsy. Inflammatory reaction on +2 day.
	−15	3.0	
	−12	2.5	
	−8	3.1	
	−5	2.7	
	−1	3.6	
	+2	5.3	
	+8	4.3	
	+15	4.7	
	+19	3.7	
683	−20	2.1	Moderate amount of tumor tissue present at necropsy. No explanation for high value on 0 day just before tumor inoculation.
	−16	2.5	
	−12	2.4	
	−5	2.5	
	0	4.1	
	+5	4.9	
	+9	3.9	
	+14	4.3	
	+21	4.0	
684	−20	2.5	Moderate amount of tumor tissue at necropsy.
	−16	2.4	
	−12	2.5	
	−5	2.6	
	0	2.4	
	+5	2.5	
	+9	3.4	
	+14	4.4	
	+21	4.2	
688	−19	3.1	Large amount of tumor tissue at necropsy.
	−16	2.4	
	−11	2.5	
	−5	2.5	
	0	2.7	
	+5	2.1	
	+8	2.5	
	+14	4.5	
	+21	4.6	

TABLE 8.—*Animals for which a diagnosis of tumor could not be made on basis of the proteose content of their serum*

Rabbit No.	Time of sample	Wave height in chart units	Necropsy record and remarks
	Days		
	−19	2.6	
	−15	2.8	
	−12	3.1	
	−8	3.1	
670	−5	3.1	Large quantity of tumor tissue at necropsy. Animal had sniffles on −1 day. Killed on +19 day
	−1	4.0	
	+2	3.7	
	+8	3.6	
	+15	4.5	
	+19	3.5	
	−19	6.1	
	−15	5.2	
	−12	5.0	
	−8	4.2	
673	−5	4.5	Large amount of tumor tissue at necropsy. No visible abnormality at start of the experiment.
	−1	4.4	
	+2	4.4	
	+8	4.5	
	+15	6.1	
	+19	4.1	
	−19	3.3	
	−15	4.6	
	−12	3.3	
	−8	2.2	
674	−5	3.4	Did not develop a tumor. Had sniffles on −1 day, and extensive coccidiosis at necropsy.
	−1	6.0	
	+2	5.2	
	+8	4.8	
	+15	3.0	
	+19	2.6	
	−18	3.2	
	−16	3.4	
	−12	2.6	
	−8	2.9	
	−4	2.7	
77	0	2.4	Moderate amount of tumor tissue at necropsy.
	+6	2.2	
	+9	2.3	
	+11	3.0	
	+20	3.0	
	+27	3.6	
	−18	5.5	
	−16	4.4	
	−12	3.3	
	−8	3.6	
680	−4	3.4	Moderate amount of tumor tissue at necropsy. Had sniffles on −18 day.
	0	2.4	
	+4	2.8	
	+7	3.5	
	+20	3.8	
	0	2.3	
	+4	2.3	No tumor and no other abnormality at necropsy. This animal and animals E and B were inoculated in each gluteus maximus muscle in addition to the usual manner.
C	+6	3.6	
	+12	5.4	
	+22	2.0	
	0	2.6	
	+4	2.2	
E	+6	4.2	No tumor and no abnormality at necropsy.
	+12	7.5	
	+22	2.2	
	0	2.5	
	+4	2.8	
B	+12	15.0	Large amount of tumor tissue at necropsy.
	+22	5.1	

The majority of the animals planted with the tumor were followed for several days before inoculation in a fashion similar to the normal group of table 6. Table 7 lists representative data on some of these. By contrast with tables 3, 4, 5, and 7, table 8 lists the seven animals that are exceptions to the general picture of relatively low normal and an elevated serum proteose after tumor development. These represent about 14 percent of the group for whom data were deemed sufficient to be included in the final tabulation. Animal No. B is included in table 8 for comparison with its serial mates C and E and for the reason that at 12 days it had the highest proteose wave height encountered for a rabbit serum.[7]

DISCUSSION

From the data it is apparent, as with the rat, that respiratory infection gives a noticeable rise in proteose. Clinically, it might be possible to rule out similar cases in making a differential diagnosis. Why rabbit No. 673 had such a large initial wave is puzzling. No respiratory symptoms of infection were noted at any time, and postmortem examination showed no abnormality other than the tumor. Occasionally an animal showed a falling off in the wave height after tumor development, as in the case of Nos. B, 661, and 662. This may be due largely to the moribund state of the animal. In many instances it was not possible to take any one value after implantation and by comparison with the average, either for that animal before, or for the group assert that the rabbit had cancer. It may be, however, that this tumor and this experimental animal are poorly suited to a study of this sort.

A portion of the data suggests that the site of the tumor may have some influence on the amount of proteose present, as in the case of rabbit No. B just mentioned, which had been implanted in both testes and both legs; rabbits Nos. 716 and 706, not listed in the tables, both cachectic and moribund at the time of sampling and necropsy, No. 716 at 30 days, and No. 706 at 47 days after implantation had

[7] The second highest proteose wave was found with the serum filtrate of rabbit 727, not tabulated herein, moribund from 2 large testicular tumors and pneumonia with consolidation of both lungs. At +16 days the value was 13, up from 1.7 on 0. day.

extensive and about the same amount of total tumor involvement except that the liver of No. 706 contained much more tumor tissue. The wave height for No. 716 was 4, and that for No. 706, 10.

It is generally believed that the increase in proteose content of the blood in the presence of the tumor is the result of increased breakdown of protein of larger molecular weight, although conclusive evidence in support of this idea has not been presented. It rests primarily on the fact that the serum proteins can be broken down in vitro by such agents as enzymes and acids to give proteoses. Instances such as Nos. C and E, in which there was no tumor but there was an increase in proteose, suggest that this increase may be part of the effort of the animal to prevent the growth of the tumor. Such instances are encountered too infrequently to permit any final judgment, but this is an alternate possibility that might be borne in mind. In this connection, the subsequent history of the animals of the N-1 to N-6 series (table 6) is interesting. Seventy-three days after the inoculation with the normal testicular material, N-1 and N-2 were inoculated with the Brown-Pearce tumor as part of the 705–710 series. At +30 days the proteose values were: N-1, 4.4; N-2, 2.85; No. 706, 5.65; and for No. 710, 4.45. At necropsy on +33 days N-1 and N-2 showed no trace of tumor while the last two had extensive tumor involvement. N-3 died intercurrently of pneumonia. Animals N-4, N-5, and N-6 were then inoculated as part of the 711–719 series 103 days after the inoculations shown in table 6. On +20 day all except N-6 seemed to have palpable tumors; biopsy was not done. The proteose values were: N-4, 3.8; N-5, 2.8; N-6, 2.6; No. 712, 4.6; No. 715, 5.2; and No. 716, 3.8. On +30 day, the proteose values were: N-4, 4.5; N-5, 2.4; N-6, 1.5; No. 712, 3.8; No. 715 was dead because of tumor; and the value for No. 716 was 4.2. At this time, N-5 and N-6 bore no palpable tumors while N-4, No. 712, and No. 716 had extensive palpable tumors in both testes and on the spermatic cords. Necropsy confirmed this. Since N-5 and N-6 seemed to be tumor-free, they were reinoculated as part of the 724–730 series 48 days after the previous tumor injection. On +17 days after this implant, the proteose values were: N-5, 2.3; N-6, 1.5; No. 724, 5.2; and No. 725, 4.0. The first two had no palpable tumors whereas No. 724 had two large testicular tumors, and No. 725 had medium ones. On the eighty-fifth day after this last injection, N-5 and N-6 were killed and found to be normal throughout. The last two were long since dead from the effects of the tumor. This partial immunity is believed to represent the usual nonspecific immune response under such circumstances plus the fact of increased age compared with their serial mates, of the later inoculations.

Examination of the polarographic waves as the work was being carried on never revealed any qualitative difference in their character, whether the animal had a cancer, pneumonia, coccidiosis, or was normal. Any difference was simply quantitative as shown by the change in height of the waves.

SUMMARY

An intensive study of the polarographic method applied to sulfosalicylic acid filtrates of rabbit serum was made. There was considerable variation in the polarographic activity for one normal rabbit at different times, but not for as short an interval as 1 day. There was considerable variation in the activity from rabbit to rabbit. Inoculation with normal-rabbit-testis mash into the testes of normal rabbits gave no increase in polarographic activity. Generally, there was an increase in polarographic activity when well-developed tumors were present following testicular inoculation with the Brown-Pearce carcinoma. Only in extreme cases was there pronounced correlation between size of tumor or metastatic involvement and activity. In those cases where there was an increase in polarographic wave height following successful implantation, this increase usually

did not take place until palpable tumors were present, presumably reflecting systemic effects from the tumor. No qualitative differences in the character of the recorded polarographic waves of filtrates of serum from normal and tumor-bearing animals were detected.

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FURTHER STUDIES ON URETHANE-INDUCED PULMONARY TUMORS

By PAUL S. HENSHAW, *senior radiobiologist*, and HENRY L. MEYER, *senior scientific aide*, *National Cancer Institute, National Institute of Health, United States Public Health Service*

Previously,[1] it was shown that intraperitoneal injections of 10-percent aqueous solution of ethyl carbamate (urethane) (1 ml. per 100 gm. of mouse) into strain A mice 6 to 8 weeks old greatly enhanced the number of lung tumors in mice killed at 6 to 12 months of age. It became of interest then to determine whether lung tumors could be obtained if the urethane was administered in other ways.

Two other methods of administration were tested. The first consisted in the subcutaneous implantation of crystalline urethane (1 mg. per gram of mouse). Strain A male and female mice, 6 to 8 weeks old when treatment started, were used. They were divided into 3 groups of 20 animals each (equal numbers of males and females being used in this and all the following experimental groups). The first group served as controls, the second received 1 implantation, and the third 3 implantations with an interval of 1 week between treatments. The animals were all killed when 7 months old, and their lungs were examined for the presence of peripheral tumors. Table 1 shows the number of tumors visible to the unaided eye after brief fixation of the lungs in Telly's fluid.

TABLE 1.—*Peripheral lung tumors per mouse receiving subcutaneous implantation of crystalline urethane*

Sex	Tumors of mice by group		
	Control	One implantation	Three implantations
	Number	*Number*	*Number*
Male	0	3	7
	0	2	11
	0	1	5
	0	2	5
	0	6	6
	0	5	10
	0	3	10
	0	7	2
	0	14	5
	1	2	6
Female	0	2	12
	0	1	7
	0	3	9
	0	1	13
	0	1	6
	0	2	7
	0	1	11
	1	2	24
	0	2	25
	0	1	17
Total	2	61	198
Average	.1	3.1	9.9

According to the second method, urethane was administered orally. The agent was dissolved in the drinking water to which the animals had free access. Two groups (10 males and 10 females each) were used. One group, kept as controls, received tap water; the other received a 0.1-percent solution of urethane in tap water, beginning when the animals were 4 weeks old. No other fluid was offered them during the remainder of their lives. All were killed at 7 months of age and examined for lung tumors. Table 2 gives the average lung tumors per animal for both control and experimental animals. No tumors were found at the site of implantation of the crystals, and none were found along the alimentary canal or elsewhere in the bodies of the animals.

In the previous studies and again in the experiments just described, the number of lung tumors observed increased directly with the amount of urethane administered. This increase suggests that the cells of the lung vary in susceptibility to urethane and that for a given dose of the agent all pulmonary epithelium having a susceptibility above a particular level responds by giving rise to tumors. Other questions then were whether the vulnerability of the cells varies with age of the host animal and whether the amount of vulnerable tissue varies with time.

In an attempt to obtain information along this line, an experiment was carried out, using 4 groups of strain A

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TABLE 2.—*Peripheral lung tumors per mouse in control mice and in those receiving urethane dissolved in tap water*

Sex	Lung tumors in—	
	Control mice	Mice receiving urethane
	Number	*Number*
Male	0	31
	0	53
	0	[1]
	0	27
	1	[1]
	1	61
	0	9
	2	31
	0	16
	1	38
Female	1	26
	0	72
	0	[1]
	0	18
	0	60
	1	62
	0	4
	0	35
	0	4
	2	40
Total	9	587
Average	.45	34.5

[1] Animal died early, not used in averaging.

mice, 20 mice equally divided as to sex in each group. Group 1 received 4 anesthetic injections intraperitoneally with an interval of 1 week between treatments; group 2 had only 2 such treatments, but they were given simultaneously with the first 2 treatments of group 1; group 3 received 2 weekly injections, but 2 months later than those in group 2; and group 4 received 4 anesthetic treatments, 2 early as in group 2, and 2 late as in group 3. In all groups except group 3, the first injections were started when the animals were 6 weeks old, and in groups 3 and 4 the late treatments were given when the animals were 14 and 15 weeks old.

All mice were killed at 8 months of age and examined for the presence of peripheral lung tumors. It was anticipated that some difference might be found in the ratio of large to small tumors. Hence it was decided to classify them arbitrarily according to

TABLE 3.—*Peripheral lung tumors per mouse after different treatments with urethane*

Sex	Group							
	1		2		3		4	
	Large tumors	Small tumors	Large tumors	Small tumors	Large tumors	Small tumors	Large tumors	Small tumors
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Male	11	1	6	7	11	11	30	42
	1	5	14	2	4	8	25	18
	11	5	20	0	2	2	16	17
	13	4	21	8	6	12	24	21
	22	4	18	12	1	8	19	7
	[1] 1	1	34	3	1	5	46	31
	8	5	12	5	4	7	3	10
	35	6	13	4	0	4	16	16
	34	8	22	4	4	9	11	15
	25	8	10	2	8	17	8	4
Female	13	10	10	3	0	4	19	31
	26	9	14	10	0	4	27	14
	32	14	12	5	2	3	5	4
	36	4	12	3	8	7	11	11
	33	7	16	5	8	7	29	25
	20	14	1	3	1	2	28	22
	11	15	11	4	4	7	12	12
	19	12	5	2	1	4	23	10
	8	6	16	4	0	5	16	12
	12	7	22	3	7	13	2	9
Total	371	145	289	89	72	139	370	331
Average	18.5	7.25	14.5	4.45	3.6	6.95	18.5	16.55
Grand total	516		378		211		701	
Average	12.9		9.45		5.23		17.5	

[1] Animal had giant spleen.

tumor size: those 1 mm. or more in diameter, and those less than 1 mm.

The results are given in table 3. Since this experiment was run concurrently with those described previously, no additional controls were used. In the previous experiment, the controls showed a tumor incidence of less than one-half of 1 percent. It may be presumed therefore that spontaneous lung tumors contributed in a very small way to the incidence observed here.

The table shows that for group 1 the ratio between large and small tumors was about 2.5 to 1, and that for group 2, about 3.2 to 1. In the latter group, the number of tumors seen was less in accordance with the number of treatments administered, but the ratio of large to small tumors remained generally about the same. In group 3, the ratio was 0.5 to 1. In this group the total number of tumors seen was less than when the two treatments were given early. The fact that the small tumors were about twice as numerous as the large suggests that the majority were small because they had not had sufficient time to become large, that is, a short growing period, and also that some expected tumors had not had time to appear. In group 4, the ratio was 1.1 to 1. Here the tumor incidence was highest.

By comparing the figures of groups 1 and 4, some idea may be obtained of the manner in which the tumors arose. Since the proportion of small tumors is much higher in group 4, it is plain that the late treatments contributed materially to the number of tumors seen. Also, since the number of tumors seen in this group is noticeably higher than that in group 1, it is suggested that the amount of vulnerable tissue may have increased with age. Furthermore, since the number of small tumors in group 3, where the treatments were given late, is somewhat larger than the number of large tumors, it may be said that the small tumors in group 1 were present because the tumors originated at a later time. If this be true, it may be deduced that the appearance of tumors takes place any time over a considerable period, actually weeks or months. This is interesting, inasmuch as the anesthetic effects of urethane wear off within a few hours after administration, and the crystals injected subcutaneously likewise disappear within a few hours.

These experiments indicate that lung tumors can be obtained as readily by the subcutaneous implantation of urethane crystals or by way of the drinking water as by intraperitoneal injection. Under the conditions of these experiments, tumors arose in the lungs only and not at the site of injection or elsewhere in the body.

The results with different patterns of treatment suggest (1) that most of the lung tumors arise within 1 or 2 months after treatment, (2) that lung-tumor tissue, which is not susceptible to malignant change early, may become so later, and (3) that some induced tumors arise appreciably later than the majority.

IMPLICATIONS FROM STUDIES WITH PHYSICAL CARCINOGENS

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Physical carcinogens may be set aside from the chemical and biologic as those involving the application of energy rather than material substances (including living organisms). Physical agents, because of their particular characteristics, permit investigations or analyses of the carcinogenic process in a manner not possible with other types of agents.

This paper (1) brings together pertinent information derived from studies with physical carcinogens, (2) considers the broad implications arising therefrom, (3) seeks to correlate the findings of similar results obtained with other carcinogens, (4) examines this body of evidence in the light of various ideas of cancer, and (5) sets forth the writer's view of carcinogenesis at this time.

STUDIES WITH PHYSICAL CARCINOGENS

The more significant physical carcinogens may be listed as mechanical irritation, heat, sunlight, ultraviolet light, and ionizing radiations (X-rays, gamma rays, neutrons, alpha particles, etc.). Other physical agents, such as radio waves, sound waves, visible light, and fields, such as electrical, magnetic, and gravitational, have not, so far as we know, been associated in any significant way with carcinogenesis. This discussion, therefore, deals only with carcinogenic changes associated with the first group of agents.

MECHANICAL IRRITATION

Chronic irritation has been associated with malignant disease since a very early period in scientific medicine. Broussais (cited by Ewing (*1*, *p. 20*)) in 1821 presented the doctrine that cancer is the sequel to recurrent inflammation. The basic features of this view persist to this day in the minds of many writers.

The skin, being exposed to the external environment, is subjected to repeated injury. Acanthoma, according to Ewing (*1*), is almost exclusively the result of traumatism, the forms of irritation being extremely varied. Basal-cell epithelioma often arises at sites of scars of indolent ulcers resulting from contusing, crushing, or lacerating mechanical injury anywhere on the body. Epithelioma of the lip and epidermoid carcinoma of the tongue and mouth have often developed in areas continually irritated by jagged teeth over a period of months or years. Likewise cancer of the lip has often been attributed to irritation caused by the stems of clay pipes. While mechanical injury may be a significant factor in such cases, the picture is often complicated by the presence of chemical compounds or organisms. Medication, mouth washes, tobacco smoke, saliva, and chemical and bacterial contamination are only a few of the foreign agents that may enter these wounds. Hence, although traumatic injury may comprise a conspicuous part of any precancerous condition, it is often difficult to be certain that chemical and biologic agents (viruses, bacteria, parasites) do not exert a significant influence on the tissue changes. In the descriptions herein, examples are presented in which physical agents clearly play a part in the carcinogenic process, but at the end the question whether these agents play such a role is still unsettled.

Let us consider first the relationship of bone injury to neoplastic growth. Bone fracture is a serious injury often requiring X-ray photographs. If tumors develop at a later time and other photographs are made, there can be little doubt as to the relationship of sites in the two cases. Furthermore, bone injuries often occur without breaks in the skin thus preventing the usual contamination and obviating the necessity of medication.

Matz (*2*), who studied 45 case histories of bone tumor in ex-service men, found that 13, or 28.9 percent, gave a positive history of trauma preceding malignant growth. In 1, there was a

definite negative history, and in 31, or 68.9 percent, the clinical records gave no evidence one way or the other. In the 13, the length of time between trauma and the first symptom of bone tumor varied from less than 1 month to 40 months. Ewing (*1*) stated that as a rule trauma preceding bone sarcoma is moderately severe and may result from fracturing or splintering and may involve local hemorrhage. Despite these observations, some have questioned the relationship between single injury and bone tumors.

The frequency of tumors generally associated with trauma is not well known. Figures vary widely with different statisticians according to their conception of trauma and their critical standards. According to Ewing (*1, p. 118*), composite statistics varied from 2.5 percent, given by Kempf, to 44.7 percent, given by Lowenthal. Ewing also gave the following figures: of 2,641 carcinomas, 107, or 5 percent, and of 938 sarcomas, 138, or about 19 percent, were classed as traumatic in origin.

HEAT

The literature gives several accounts for the period 1800–1850 describing cancer which arose at the site of scars resulting from burns. In 1828 Marjolin (*3, p. 22*) published his classic description of ulcer that originates in degenerate scars and is known today as Marjolin's ulcer. More recent reports of cancer at the site of burns are those of Johnson (*4*), Treves and Pack (*5*), Arndt (*6*), Danzis, Friedman, and Levinson (*7*), and Harkins (*8, p. 177*).

The material by Treves and Pack is complete with case reports and summary figures. During the period 1917–29, inclusive, 1,091 patients with epidermoid carcinoma of the skin, and 1,374 with basal-cell carcinoma were treated at Memorial Hospital, New York. In 28, the cancers developed at the site of old burns; 21 of them were epidermoid carcinomas, and 7 were basal-cell epitheliomas. On the basis of these figures Treves and Pack estimated that about 2 percent of all epidermoid carcinomas and about 0.3 percent of all basal-cell carcinomas originate on skin subjected to thermal injuries. The interval between injury and onset of malignant disease varied from 1½ months to 63 years. In 6 cases, this interval was 1 year or less. However, with the exception of 1 patient, who gave a history of repeated injury to the site before the burn, the patients having a short latent period were 53 years of age or over at the time of injury. Although some of the remaining 22 patients gave a history of previous injury to the site of the burn, the average latent period was 30 years, the shortest being 7. Three of the patients were 3 years old when burned. One was 7 months old when a severe scald was received on the scalp; 37 years later a white, hairless scar, which had peristed during this time, developed into a progressive ulcer. Most of the patients had a history of poor healing or repeated injury due to contraction and fissuring, accompanied in some cases by neglect, poor care, and unwise medication. Of the 28 patients with carcinoma following burns, not 1 was treated by skin graft.

Arndt's account deals with 99 cases, including some of those described by Treves and Pack. Arndt reports 1 case of his own, which is typical of the series: A woman 44 years old was burned at the age of 3; no skin grafting was done, and the wound was open off and on for 30 years. In Arndt's series, the latent interval was known in 81 cases; the average was given as 32.8 years. Nineteen of the cases were of the acute-wound type and followed the burn by an average of only 11 months. But again these wounds were among older people, whose average age at the time at the time of the burn was 48.7 years.

The reports concerning the kairo oven (*5*) and kangri fire bowl (*9*) are of particular interest since they indicate a close relationship between heat as an injurious agent and the formation of skin cancer.

The Japanese use the kairo to keep the body warm. The device consists of a light metal box 7 x 4 x 1 inch, with a sliding top; the box is curved on one broad surface to fit the contour of the

abdomen. It is charged with powdered charcoal, which burns slowly after being ignited, and is worn in a belt (often richly brocaded) under the kimono and against light undergarments. The heat generated by a single charge may last 3 hours or more. The kairo is worn particularly by elderly women while attending religious ceremonies in cold temples. Cancer often develops where the kairo rests against the body.

The kangri is used by the poorer class of Kashmir, northwestern India, for the purpose of keeping the body warm. It consists of an earthenware bowl 5 or 6 inches in diameter surrounded by wicker basketwork with a handle. It is worn or carried against the body under a single, loose, smocklike garment, and it is also placed between the thighs when the individual is seated on the ground with knees drawn up against the chest underneath the garment. Neve (*9*, *10*) reported that the temperature of the skin against which the kangri rests may be 150° to 200° F. in normal use of the fire basket. Scars from kangri burns are frequent, and the wounds that have developed are often badly neglected. Cancer at such sites is common; the average age of the persons affected is 55. A few cases of cancer from kangri burns have been seen in patients under 40, but they have been rare. About 6 to 7 percent of kangri cancers are in persons over 70. According to Neve, there may be some predisposing factor. Many elderly Kashmiris exhibit small, localized papules or macules, which are dry, slightly scaly, and pigmented. Where there is actual exposure to heat, every stage of chronic dermatitis may be seen, from redness with or without desquamation to thickened patches of warty induration or even horny outgrowths from the surface. The skin of the thighs and the abdomen, because of the constant application of heat, often appears dry and horny. Pigmentation is increased over the distribution of the superficial veins, the course of which is marked by brown discoloration. Such persons are especially prone to epitheliomas. The frequency of actual scars from previous burns is noteworthy, and it is these scars that usually form the starting point of malignant growth.

Neve stated that from 1881 to 1923, there were 2,494 operations performed at the Kashmir Mission Hospital, and that of these, 2,000, or 84 percent, were for kangri cancer.

Of interest in this connection is the brief report cited by Hueper (*11*, *p. 293*) on kang cancer. The kang is a brick platform across one side or end of the room, found in northern China and Manchuria. It is used as the sleeping place for the whole family. In the winter it is heated from the inside. Burn-scar cancers of the hip develop as a result of resting on the heated surface. Hueper speaks of these as "apparently genuine burn scar cancers" and states that the kangri and kairo cancers may be classified more appropriately among the soot or tar cancers.

It is a little-recognized fact that sarcoma may also develop at the site of burn scars. "Epithelioma-malpighien sarcomatoïde" on an arm of a 37-year-old man was reported by Grynfeltt and Aimes (*12*). The burn had been received 30 years previously, and the wound had never completely healed. Harkins (*8*) reported two cases. The first was a 67-year-old woman who died of recurrent tumor of the skin of the right thigh. At the age of 10 she was severely burned when her clothes caught fire and as a result was kept in bed for 2 years. The healing time was not given, but skin grafting was never done. Eleven years before her death, the scarred area became ulcerated and then intermittently broke down and healed until the time of death. A diagnosis of sarcoma was made. The second case was a girl who was severely burned on the chest and the right breast when she was 10 years old. Because of keloid formation, roentgen therapy was administered and plastic surgery was performed. Seven years later, she died of sarcoma of the right breast.

Apparently little has been done concerning the experimental induction of tumor at the site of a burn, although Bang (*13*) reported positive results in 2 animals. Twenty-two mice were

burned with hot glass rods over a large area on the back which had been shaved. The wounds all healed in about a month; 11 showed some epithelial thickening. Fifteen animals were dead at 6 months, but 5 lived 1 to 2 years; 1 of these developed a papilloma that changed to carcinoma. In a second series, 5 young mice were given the same treatment. Healing occurred as before in about 1 month, although in 1 animal ulcer developed in the scar at 3 weeks and by 9 weeks became precancerous.

The following statement on burn injury is quoted from McClure and Harkins (*14*), of the Henry Ford Hospital: "Carcinoma: this late complication, while rare, practically never develops in a rapidly healed burn no matter how extensive. It is the result of neglect."

SUNLIGHT

Sunlight as a possible causal agent in skin cancer has long been recognized. Unna (cited by Blum (*15*)) described seamen's skin as a precancerous condition attributable to continued exposure to light. Blum (*15*) points out that there were several early exponents of this view (Dubreuilh (*16*, *17*), Sheild (*18*), and Bellini (*19*)) and states that while at present few writers oppose the idea that sunlight may be an etiologic factor in cutaneous cancer, opinions vary regarding its relative importance.

Blum (*15*, *p. 397*) summarizes the conclusions of various workers as follows:

> (1) Cancer of the skin occurs principally on parts exposed to sunlight; (2) cancer of the skin is more prevalent in outdoor workers; (3) the incidence of cancer of the skin is greater in regions of the earth which receive the greatest insolation; and (4) cancer of the skin occurs more often in blonds than in brunets.

Mackee and Cipollaro (*20*) give a description of "farmer's and sailor's skin" on exposed areas of adults, mostly middle-aged and elderly persons, who have been exposed to the sun for many years. The skin becomes wrinkled and dry, showing permanent lentigo, telangiectasia, and white, sclerotic spots. The lips are apt to become dry and fissure easily. Keratoses of the senile type frequently develop in such skin. The condition bears some resemblance to chronic dermatitis. Epithelioma, often of the squamous-cell type, develops commonly from "farmer's and sailor's skin." Preceding the onset of active growth, degeneration and atrophy of the connective tissue occur in the upper layers of the true skin. The epidermis is somewhat atrophic except in areas where keratoses are developing.

Observations such as those just described have led to considerations and experiments with ultraviolet light.

ULTRAVIOLET LIGHT

The first experiments dealt with the question whether tumors could be induced in animals with ultraviolet light. Several workers (Findley (*21*), Putschar and Holtz (*22*), and Herlitz, Jundell, and Wahlgren (*23*)) undertook experiments apparently independently and at about the same time, and all obtained some degree of success. Work along this line was undertaken by others, including Blum, who has carried out the most extensive studies.

Blum (*24*) giving repeated exposures five times weekly to mice was able to induce tumors on the ears in 100 percent of the animals. Both carcinomas and sarcomas were obtained. The interval between the beginning of treatment and the appearance of tumors was shortened (to 3 or 4 months) by increasing the daily dose up to a certain limit. Increasing the dose beyond this point produced no further change in the induction time. Other experiments showed that if the treatment schedule was interrupted or stopped, the induction time was longer, and that it varied inversely with the number of treatments applied.

Blum and his associates (*25*) were able to obtain tumors with only slight evidence of precancerous lesions. They pointed out that with large daily doses the tissue destruction preceding tumor

formation might be severe, whereas with small daily doses it might consist of no more than slight hyperplasia with no skin abrasions or ulcer. They stated that "it is difficult to say that hyperplasia is an essential factor for tumor formation, or even that the two processes are directly related."

IONIZING RADIATIONS

The discovery of roentgen rays was announced in December 1895. Reports are on record of irradiation injury within weeks (Marcuse, cited by Porter and White (*26*)). In 1902 Frieben (*27*) described what appears to have been the first case of roentgen carcinoma. This carcinoma developed on the back of the hand of a young man employed in the manufacture of X-ray tubes. At that time it was customary to test for the presence of radiation by viewing the bones of the hand with a small fluoroscope. Because of the soft character of the rays first used and the short distances involved in the handling of radium, most of the earlier injuries were confined to the skin. Later, however, other malignant diseases, notably bone sarcoma and leukemia, were associated with exposure to ionizing radiations. Since the nature of irradiation injury and precancerous irradiation ulcers have been extensively studied and since each of these types of tumor shows features that have a significant bearing on the question of tumor induction, examples are given.

RADIOCARCINOMA

Vivid accounts are found in the literature of carcinoma arising from X-ray injuries. The early case reported by Porter and White (*26*) is typical:

An X-ray operator as a young man began work with a large static machine in March 1896. After October of the same year, a "powerful" 12-inch coil was used. Exposure lasted several hours each day, at a short distance from a low vacuum tube. About the middle of November, his hands became red and dry and in a few days were painful and greatly swollen. His finger nails first became dry and striated, then softened, and finally sloughed off. In spite of intense pain, he continued work until April 1897, when he had an onset of another severe dermatitis, accompanied by the production of enormous blisters and further pain. In July 1897 grafts were applied to the tip of his left forefinger which had the appearance of sluggish ulcer with exuberant granulation and unhealthy-looking edges. This graft took, and the lesion improved. In August 1897 all the ulcerated areas were cleared and 14 different grafts applied. The grafts adhered, and healing took place. Pain ceased for a period; but in time other ulcers appeared, and the pain returned. In 1902 biopsy specimens showed unmistakable carcinoma. Treatment in this case, like that in many others since, ran a tragic course of excision, grafting, and amputation.

Wolbach (*28*) in 1909 used excised tissues from persons with late dermatitis and made a careful study of the histologic changes therein. Comparing the tissues of normal skin with those of lesions approaching the cancerous state, he described four types of modifications: (1) A thickening and downgrowth of epithelium; (2) a rarefaction of the loose connective tissue immediately beneath the epidermis, characterized as being in a state of imperfect repair; (3) vascular changes, consisting mainly of telangiectasia, loss of elasticity, and obliterative endarteritis; and (4) atrophy of the smooth muscle, mostly in blood vessels. Wolbach stated that in general the increased thickening of the epidermis and the production of wartlike growths with keratoses and downgrowth are best explained as the result of constant, active, proliferative repair called forth by the continuous occurrence of small defects in the underlying corium. In a later paper (*29*) Wolbach stated that "the outstanding factor of importance is that conditions are continually occurring which call forth proliferative activity of the epidermis greatly in excess of the rate of normal proliferation" and inferred that the acquisition of malignant properties is not sudden

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but is gradual during the course of years.

The histologic changes in the skin and underlying tissues following exposure to ionizing radiations have been worked out in some detail; a review of this work is given by Montgomery (*30*). Although much has been added in the way of minutiae, very little has been added regarding the understanding of which tissue condition is cause and which effect.

The findings of Saunders and Montgomery (*31*) are typical of the kinds of irradiation injury seen in recent years. These authors reported 259 cases of radiodermatitis, which were seen at the Mayo Clinic during the 5-year period 1930-34, inclusive. It was found that the large majority of irradiation injuries were contracted through injury to the patient during therapeutic exposure rather than occupationally as in former years. Twenty-six (10 percent) of the patients were physicians. Except for the hands, in the case of the physicians, there was no predilection for particular sites. The time from initial exposure to the first signs of injury varied from 6 months to 18 years, all cases of acute or immediate injury being excluded. Aside from the physicians, 14 percent of the patients were injured during treatment for malignant, and 86 percent during treatment for nonmalignant diseases. Epitheliomas developed in 27, or approximately 11 percent; they were of the squamous-cell type and appeared from 1 to 11 years after the first signs of dermatitis or in 1½ to 20 years after exposure to the radiation.

Radiosarcoma

The cases of radiosarcoma among the New Jersey watch-dial painters, reported by Martland (*32*), are excellent examples of this type of disease. Young women employed for this work made a practice of pointing the fine paint brushes with their tongues, thus ingesting appreciable amounts of radium element. Most of the radioactive material passed rapidly through the gastrointestinal tract and was eliminated, although a small amount was continually absorbed and eventually deposited in the reticulo-endothelial system and particularly the bones.

Martland stated that it is necessary to have only 10 micrograms of radium (radium bromide) distributed over the entire skeleton to produce death in 5 to 15 years. Quoting a personal communication from Gettler, he reported that the bones of a dial painter exhumed 5 years after death for purposes of litigation were found to contain approximately 50 micrograms. In this connection, it is interesting that Rajewsky (*33*) reported that 1 microgram of radium deposited in the body causes severe injury, that the National Bureau of Standards Handbook H–27 (*34*) gives the tolerance limit as 0.1 microgram, and that Krebs (cited by Rajewsky (*33*)) found the human body in middle life to contain on the average approximately 0.01 microgram. (His figure is 7.5×10^{-9} gm.) These amazing figures indicate that roughly there is a tenfold difference between the lethal, the injurious, the tolerance, and the normal level.

Martland reported 18 fatalities among dial painters, 4 of whom had primary periosteal osteogenic sarcoma, and 1 who had primary periosteal fibrosarcoma. The causes of death in the others were ulcerative stomatitis, anemia, pernicious anemia, regenerative anemia, aregenerative anemia, necrosis, etc., diseases or conditions which bear an interesting relationship to osteogenic sarcoma and leukemia. The dial painters who later developed bone sarcoma were employed from 15 months to 5 years. The interval between the beginning of employment and the diagnosis of sarcoma was 5 to 13 years, and death usually occurred within a year after diagnosis.

Martland made a detailed study of the roentgenologic, hematologic, and histologic changes that appeared progressively in a number of persons who developed osteogenic sarcoma. Histologically, the bone marrow showed a picture quite unlike that seen in any other disease. The normal architecture was entirely obscured by an extreme

hyperplasia and crowding of the bone marrow with immature and primitive cells.

Three stages were recognized. In the first stage, the marrow was described as "hyperplastic" and "irritative" and was said to contain a great number of eosinophilic myelocytes. The primitive stem cells appeared to have lost their ability to form other cells of the granulocyte series. The hyperplasia was interpreted as a compensatory stimulation caused by the radiation. The second stage was referred to as "replacement fibrosis." At this stage, young fibroblasts grew across and into the hyperplastic marrow. Such areas could be seen grossly as distinct, grayish-white fields, usually reflecting high lights and ranging in size from scarcely visible to 1 to 2 cm. in diameter. Microscopic sections showed many eosinophilic myelocytes, plasma cells, and lymphocytes. Mitotic figures were common, with hyperchromatism. Areas of replacement fibrosis gave rise to sarcoma, and there appeared to be no sharp line of demarcation between replacement fibrosis and sarcoma. The third stage was one of healing, with almost complete subsidence of all inflammatory reactions. Here the marrow was replaced by a relatively acellular fibroblastic tissue. By the time this stage was reached, bone absorption and considerable decalcification had taken place. The bones at necropsy felt soft and woody when sawed through and showed grayish-white sclerotic areas in the marrow. The consequence of these changes was the softening of the bone, which resulted in spontaneous fracture or other deforming lesions.

The three stages are covered by the term "radiation osteitis." The picture Martland presents suggests that through the effects of ionizing radiations a hyperplastic marrow is developed and that this in turn progresses into a condition of abortive repair which may turn either into neoplasia or fibrosis.

Radioleukemia

Damage produced in the blood and hematopoietic organs by ionizing radiations was noted and studied at length by Heineke (*35*) as early as 1903. Heineke, working with animals, showed that with relatively small doses of radiation marked parenchymatous cell loss is produced in the lymphoid organs and bone marrow and that, if the damage is not too severe, regeneration with hyperplasia may proceed over a period of time. Occasionally, leukemia has been found in persons exposed occupationally or otherwise to ionizing radiations, and because of the similarities between cycles of injury, repair, hyperplasia, and neoplasia seen in the skin and in the hematopoietic organs, observers have been quick to comment on the possible etiologic significance of radiation in leukemia.

Dunlap (*36*) found 24 cases of leukemia in radiation workers reported in the literature. The 24 victims ranged in age from 29 to 53 years. All had had years of occupational exposure to radiation, and several showed radium or roentgen burns on their hands. In 4, leukemia followed exposure to radioactive substances; and in the remaining 20 it developed in roentgenologists, radiologists, and their assistants, who presumably had been working with X-rays. In 7, the condition was diagnosed as lymphatic and in 13 as myelogenous leukemia, and in 4 the type was not specified. The disease in these patients did not differ in character and course from spontaneous leukemia.

Somewhat stronger evidence that radiation may bear a causal relationship to leukemia comes from experimental work with animals. Krebs, Rask-Neilsen, and Wagner (*37*) were the first to study this problem. They treated mice with X-rays and found the incidence of lymphomatosis among 3,500 irradiated animals to be 3.5 per thousand and that among nonirradiated controls to be 0.6 per thousand. Furth (*38*) gave whole-body treatments of 400-r single exposures at intervals of approximately 1 month and found an increase in leukemia (myeloid and lymphoid) of more than threefold. Hueper (*39*), using hard X-rays, gave general-body treatments to mice, doses up to 400 r being

given in a 6-week interval, and he observed an incidence of leukemia as high as 74 percent (in animals which he stated had a high but unknown normal incidence of leukemia). Furth and Furth (*40*) gave single or repeated doses of 200 to 400 r of X-rays to the whole body of mice and observed an increase in myelosis of eightfold and in mediastinal lymphomatosis of sevenfold. The actual incidence of myelosis and lymphomatosis, however, did not exceed 7.6 and 13.9 percent, respectively. In our own work (*41*), in which a critical study of dosage in the induction of leukoses was made, increases likewise were found which in some cases exceeded any others thus far reported. Henshaw and Hawkins (*42*), assuming that physicians are exposed more to ionizing radiations than is the general population, carried out a survey which indicated that the incidence of leukemia in physicians is approximately 1.7 times higher. These authors stated that while the findings furnish no positive proof that radiation acted to incite leukemia, they are, nevertheless, in accord with the results in experimental animals. More recently, March (*43*) found the incidence of leukemia in 173 radiologists to be 10 times greater than in physicians generally.

Leukemia following Trauma

Leukemia following trauma could have been presented appropriately under the heading Mechanical Injury, but is of greater interest here, following the discussion of bone sarcoma and leukemia. In a good many instances, leukemia followed trauma so closely that the possible relationship of injury to this disease was suspected by numerous observers. In 1930 Lewsen (*44*) accumulated a list of 40 such cases reported in the literature and added 1 of his own. Others have been reported since (Yaguda and Rosenthal (*45*) and Brown (*46*)).

The case reported by Lewsen is typical:

While removing an automobile tire, a man 56 years old received a violent blow on the shin from a heavy hand tool which caused an abrasion 1 inch long but no fracture of the bone. The wound was dressed; and he resumed work the next day when a second blow was suffered at the site of the first. He was unable to continue work and about 3 weeks later began to complain of fatigue, lassitude, and palpitation. During the week that followed, the symptoms got worse, and the man was confined to his bed. The leg wound, though painful most of the time, was never septic and healed in about 1 month. Frank myelogenous leukemia appeared, and death occurred 52 days after the initial injury.

On the basis of the 41 cases, Lewsen found that (1) the majority were males, (2) the average age was 38, (3) the previous history was one of good health, (4) the family history was negative, (5) the types of trauma were blows to long bones and ribs, blows in the region of the spleen sometimes causing rupture, or general severe shaking, (6) the trauma was sometimes repeated, (7) the period between injury and onset of the disease was from a few days to 4 years, (8) the leukemia was nearly always myeloid, (9) death followed the injury in 3 to 5 years, and (10) there was often pain at the site of the trauma.

Combined Effects of Physical and Chemical Carcinogens

The combined action of the various kinds of carcinogenic agents has been studied. Mottram (*47*, *48*) observed the effects of benzpyrene and ionizing radiations used together. This investigator showed that painting the skin of mice twice weekly with 3, 4-benzpyrene for 6 weeks caused some reaction of the skin but no tumors to develop. However, with continued painting or the application of a single heavy dose of beta or gamma rays, an appreciable number of tumors was induced. Similar results were obtained by Mayneord and Parsons (*49*), who used X-rays and benzpyrene in one case and dibenzanthracene in another.

Des Ligneris (*50*) combined methylcholanthrene and heat (scalding) in the same skin area. Large numbers of

animals were used, and a 0.3-percent solution of methylcholanthrene was painted on the backs of mice 3 times weekly for 3 months. At the end of this time, some of the animals had definite epitheliomas, some had transitional lesions, others had benign papillomas, and still others had no lesions. The 119 animals with benign papillomas were separated from the rest and divided into 3 groups. The first group received further methylcholanthrene treatments 3 times weekly for an additional 3 months, the second group received mild scalding at the site of painting 3 times weekly for the same period, and the third group was left alone. Fifty-two and forty-three percent, respectively, of the animals in groups 1 and 2 developed epitheliomas, whereas only 10 percent of those in group 3 did so. Other animals treated by scalding alone developed no tumors.

Des Ligneris summarized in part as follows (*50, p. 43*):

> The production of skin tumors in mice with * * * cancer-producing hydrocarbons is always preceded by a precancerous state, papillomatosis. When, as a consequence of the application of the cancer-producing chemical, the cell has reached a certain stage of constitutional alteration (though this stage may not necessarily be accompanied by microscopically recognizable changes), cancerization may proceed without the aid of further specific carcinogenic action. Nonspecific irritation (scalding) has at this stage the same effect as the specific action by a cancer-producing compound.

SUMMARY OF FACTS OBTAINED WITH PHYSICAL CARCINOGENS

The principal findings from studies with physical carcinogens[1] are as follows:

(1) A significant body of evidence is brought together which suggests that neoplasia may be induced in various types of animals and under a wide variety of circumstances without the aid of exogenous chemical carcinogens or living organisms. As indicated previously, the possible contamination with small amounts of cell-stimulating chemicals or viruses could not be excluded in most of the cases cited, especially carcinoma arising in surface wounds. However, such cases as osteogenic sarcoma following bone fracture or radium poisoning make it clear that if chemical stimulators or viruses were instrumental in the induction of neoplasia, they must have been endogenous.

(2) Injury of some kind was common to all the cases cited, and so far as we know, there was no case (with the possible exception of ultraviolet light (Blum)) in which neoplasia was induced with a physical agent without some injury first.

(3) As a consequence of mechanical irritation, regeneration and repair were common to all cases of tumor induction with physical carcinogens. It is noteworthy that various writers who dealt with thermal and irradiation burns emphasized that carcinoma is a late complication developing in wounds long-neglected and improperly healed. From this it may be deduced that long-continued stimulus for repair is often a forerunner of neoplasia. It was emphasized repeatedly that skin grafting in refractory wounds is an effective method of preventing the occurrence of carcinoma. Satisfactory healing not only stops the stimulus for repair, but presumably in some cases the stimulus for carcinogenesis is also stopped at the same time.

(4) The element of time was a conspicuous feature in nearly all the descriptions given, yet the interval between injury and onset of neoplasia varied from a few weeks to more than 60 years. It would seem, therefore, that whereas time is an important factor in carcinogenesis it is not a critical one.

(5) In general, the production of tumors with physical agents appears to follow the pattern of injury, repair, regrowth, and neoplasia.

FINDINGS WITH CARCINOGENS OTHER THAN PHYSICAL

The induction of tumors with chemical carcinogens appears to follow the

[1] Tumor induction with other types of physical agents (e. g., gall stones, ill-fitting bridles and harness, freezing and thawing, etc.), might be presented; however, it is not included in this report.

same steps in many instances. In others, chemical carcinogenesis appears to follow a different pattern. One example of each type is given herein.

Orr (*51*) prepared paraffin pellets, of which some were plain, others contained noncarcinogenic hydrocarbons, and still others carcinogenic hydrocarbons (for example, methylcholanthrene). These pellets were implanted subcutaneously in animals, and sections were made at different times to determine the course of the reaction in each case. The plain pellets and those containing noncarcinogenic hydrocarbons were found to elicit the usual foreign-body reaction leading to enclosure of the pellet with fibrous connective tissue in 2 to 3 months. After this, the reaction was complete, and there were no further changes. Pellets containing carcinogenic agents prevented satisfatory encapsulation. There was an inadequate formation of fibrous material, and the collagen formed was small in amount and loose in texture. The connective tissue beyond the point where the capsule was expected to form showed evidence of irritation and remained in a mild state of inflammation. After some months, foci of secondary cellular proliferation appeared beyond the primary collagen at a distance from the pellet, and it was from these that sarcomas developed. The author suggested that the presence of the carcinogen tended to restrict the usual foreign-body reaction, thereby preventing an effective and satisfactory end point from being reached. The surrounding connective tissues being held under a constant stimulus for repair eventually gave way to neoplastic growth. These results, in common with those obtained with physical agents, showed a pattern of mild injury, long-continued stimulus for repair, and eventual neoplasia.

The second example of chemical carcinogenesis concerns the anesthetic agent, ethyl carbamate (urethane). The author (*52*) showed that a single anesthetic dose of this agent injected intraperitoneally produced multiple pulmonary tumors in strain A mice in less than 4 months, no tumors of any kind being found elsewhere. A systematic study of the tissue changes preceding the appearance of tumors revealed no evidence of tissue injury. The reaction in this respect was not unlike that involved in the induction of breast tumors with excessive amounts of estrogen, and of fowl tumor with virus agents. The pattern of carcinogenic action in these cases appeared to involve neither injury nor repair. The cells underwent a direct transformation from the normal to the neoplastic state and sometimes with great rapidity. (Measureable tumors can be produced in 1 weeks' time with fowl-tumor virus.)

It is significant that multiple lung tumors were obtained. This fact alone makes it clear that the neoplastic growths did not arise from a single cell and that a number of cells must have undergone malignant transformations in the same way at about the same time. From the histologic study of carcinogenesis in breast and other tissues (especially mixed tumors), one also gains the impression that the same change takes place in many cells in the same way simultaneously.

SIMILARITY OF EMBRYONIC DIFFERENTIATION AND CARCINOGENESIS

A most important advance in embryology was the development of the concept of "organizers" (*53–55*). The primary organizer of the amphibian embryo is situated in the dorsal lip of the blastopore. If a fragment of this region be implanted in the blastocoele cavity of another embryo, the overlying ectoderm, though distant from the normal embryonic axis, develops into neural folds. As a result, there are two embryonic axes, and in favorable circumstances two embryos will develop. By this process of induction, the ectoderm is impelled to form structures which it would not form in normal development. When the action of the primary organizer has ceased, organizers of the second, third, and higher grades continue to regulate differentiation so that neighboring tissues develop in harmony.

Organizers exert an induction action only on tissues which are in a receptive state known as "competence." Induction starts the process of determination whereby the fate of a region is decided, and its competence or potency is lost.

Two kinds of induction have been distinguished, namely, "evocation" and "individuation." Evocation is not specific; it starts the process of differentiation but does not direct it qualitatively. Evocators have been extracted from adult tissues of all phyla. According to Needham (*56*), the active principle is probably a steroid belonging to the group which includes the sex hormones and carcinogenic hydrocarbons. Individuation is specific, it directs the course of differentiation qualitatively and determines the specific character of different organs and tissues. In carcinogenesis, agents such as methylcholanthrene and X-rays appear to act as evocators as they merely initiate the process which leads to carcinogenesis. Virus agents and urethane, on the other hand, may be regarded as individuators since they appear to impel the action of a cell or cell group. Individuator agents may even participate continuously in the process. In the same sense, Rous (*57*) has described virus action as "actuating" and that of other chemical carcinogens as "provocative."

Needham summarizes embryonic determination thus:

> * * * the prospective potency of a given part is much wider than its prospective significance. What it can do if it must is a great deal more protean than what it will do if left to itself. It has a versatility which, in the absence of experimental interference, is never shown to the world. But this versatility is not conserved throughout development. On the contrary, a restrictive rigidifying process takes place which allots to each part its destiny and after which no alteration is possible whatever new environment experimental interference may provide.

It is important to recognize, however, that while progressive differentiation of cells is accompanied by a progressive loss of potencies for further development, the two processes are not always parallel. It is difficult to know whether the particular quality a cell seems to have is finally fixed; for there may be new conditions, not yet tested, under which other potencies would be expressed.

Weiss (*59*) points out that in certain types of cells, "the latitude of physiologic adaptations may be far in excess of the relatively narrow range of variations exemplified by the chromatophore." He says (*59, pp. 93–94*):

> This holds especially true for cells of the mesodermal kind. For instance, it is an established fact that a spindle-shaped, sedentary, fiber-producing cell of the reticular tissue can react to certain pathological events in the body with very startling transformations. Such a cell can suddenly be mobilized, lose its connections with neighboring cells, become plump, amoeboid, and phagocytic—in other words, assume all the morphological and functional properties which are exhibited by free macrophages. Conversely under certain other conditions, free macrophages can settle down and transform into fixed tissue cells of the spindle-shaped type. The whole series of gradations between the two extremes is known. Differentiation of this type of cell, therefore, involves, among other things, a capacity of the cell to react to one set of conditions by assuming shape and function of a fixed tissue cell, and to another set of conditions by assuming aspects and functions of a free blood cell. Apparently the same cell can commute back and forth between the two stages, according as the conditions vary.

Weiss calls this physiologic, nonprogressive function of a cell in response to its environment "modulation," and says (*59, p. 94*):

> It provides for a certain latitude within which a cell can comply adequately with certain variable functional demands of the developed body. Both character and range of modulation are strictly circumscribed by the specific constitution which the cell has acquired in the process of differentiation.

He also points out that the criterion by which we are to tell modulation from differentiation is entirely empirical.

> It is based on the fact that modulation is a physiologic process *reversible without residue*, allowing a cell to return exactly to the condition in which it was before; while differentiation is progressive and involves changes in the cellular constitution which amount to either permanent gains or permanent losses (*59, p. 97*).

He likens the process of development, growth, and maturity to a system of

channels having a common center, the undifferentiated cell. Branching of the channels as they leave from a common center is similar to differentiation. The width of the channels represents the amount of latitude, or modulation, left to the cells.

In the normal course of development, cells appear to undergo an orderly and progressive differentiation, from which there is no return. Since certain of the changes are maintained although the environment may go back to a former state, the cells may be regarded as intrinsically changed. The nature of the change which accounts for differentiation is not understood, although it is clear that the receptor nature of the constitutional elements of the cell must be modified. Which channel of differentiation a cell enters at any particular moment is determined by environmental factors such as position, relation to other cells, and chemical concentration of substances (organizers, etc.). The capacity for further differentiation depends upon the channels that have already been entered, in other words, the residual cell potency or competence. Erythrocytes or cornified epithelium, for example, would not be expected to differentiate into any other type of cell as they have followed to the end of the channel system; they have no further competence; they do not even proliferate.

For our purposes here it is sufficient to know that orderly, progressive changes can take place in a gradual, stepwise fashion in many cells in the same way and at the same time. Development and indeed life processes in general appear to consist of a series of adaptive changes, only part of which are reversible.

VIEWS REGARDING THE UNDERLYING CANCER PROCESS

Although clinically cancer has many manifestations, thus making it necessary that the different kinds of growth be treated as different diseases, and although there is a wide variety of causal agents, it has long been believed that cancers are only individual expressions of a single, strongly characterized, neoplastic principle. Views regarding this principle have varied widely. Cohnheim (*60*) in 1887 suggested that cancer arises from "embryonic rests," that is, groups of embryonic cells which remain dormant during development and later flower into malignant growth. Boveri (*61*) later set forth the idea that cancer cells are cells with abnormal chromatin complexes derived from multipolar or other abnormal cell division. White (*62*) referred to cancer as a mass of cells, tissues, or organs resembling those normally present in the body but arranged atypically and growing at the expense of normal tissues without serving any useful purpose at the same time. Murphy (*63*) referred to cancer as a break in the control mechanism of the cell, a loss of body control of cell activity. Waddington (*64*) and Needham (*65*) suggested that fields of individuation are the points from which cancer "escapes." Lewis, according to Reimann (*66*), called cancer a new race of cells. Gye (*67*) regarded cancer as being due to a living, intracellular virus. Ewing defined cancer as an autonomous new growth of cells. Reimann (*66*) described malignancy as a transmissible, irreversible change in the composition of the cell involving its potencies of differentiation and organization. Potter (*68*) regarded cancer as resulting from the introduction of an abnormal or foreign protein called "cancer virus" which competes with a resident enzyme for substrate materials.

Many other descriptions of cancer could be listed, but these are sufficient to indicate the variety of views and the trend of thought. Cancer cells for the most part are considered to be abnormal cells, cells which escape control by the host organism, cells which are lawless or anarchistic. The question is raised whether there is any single fact regarding cell behavior, about which we now know, that requires that this general view be taken. No characteristic of the cancer cell has yet been found which does not also apply to certain normal cells. From experimental embryology, it is recognized that cells, primitive as

well as the more adult, possess various prospective potencies and that these become expressed in response to various external stimuli. Is there any need then for considering cancer cells in any other light than that in which normal cells are considered?

CANCER AS A FORM OF DIFFERENTIATION

Perhaps the most outstanding single finding in cancer research is the negative result indicating that no method has been discovered whereby a cancer cell can be distinguished with certainty from a normal cell. While it is true that conditions such as metabolic action, enzyme behavior, chemical composition, and invasiveness have been found which characterize most of the cancer tissues more strongly than most normal tissues, enough exceptions have been found in each case to prevent any of these features from being regarded as characterizing the underlying neoplastic principle exclusively.

Two other important points are: (1) There is no sharp line of demarcation between benign and malignant tumors; and (2) it is a recognized fact that distinctly nonmalignant, precancerous lesions change progressively and eventually become frankly malignant tumors.

Thus, since we know from experimental embryology that progressive cell changes take place under the influence of various physical and chemical stimuli, and from carcinogenesis that such changes also take place under the influence of various physical and chemical agents, it seems not inconsistent to think of neoplasia and development in the same terms and to think of both as being motivated by similar mechanisms. We therefore submit as a working hypothesis the idea that cancer is a form of differentiation, differentiation at a tangent or beyond the usual limits. Let us call it tangential differentiation to distinguish it from the usual or normal. With this view, it is natural enough to regard various carcinogenic agents as evocators or individuators of cell behavior. The physical and chemical evocators then would be regarded as agents that injure tissues in such a manner as to cause the elaboration of individuators or actuators, which in turn elicit neoplasia. Indeed, there is growing evidence that the body normally contains actuators of carcinogenesis, even aside from hormones, which when concentrated may be used experimentally to induce tumors (Lacassagne (*69*)).

Steiner (*70–72*), Menke (*73–74*), Kleinenberg, Neufach, and Schabad (*75*), and Aptekman, King, and Lewis (*76*) prepared benzol extracts of various normal tissues (liver, lungs) of tumor-bearing and nontumor-bearing persons or animals and found some of these to have carcinogenic properties. It would be interesting to determine whether such agents are present in greater abundance in wounds, especially the chronic type. Certainly there is no question but that wounds contain an influence that actuates changes in cell function, some of which are irreversible (e. g., formation of scar tissue). Of course, not all injurious agents induce carcinogenesis; likewise, not all stimulating agents induce accessory neural folds. Whether an induction is obtained, as is made so clear in embryology, depends upon the competence of the cell and the specific nature of its environment.

For those who hold that carcinogenesis must take place suddenly, as in the case of mutation of the genes or sudden contamination with an extrinsic agent, the lack of clear distinction between malignant and nonmalignant cells and also the fact that orderly progressive changes toward greater malignancy occur in many cells simultaneously, are very troublesome. But if we think of malignancy as a further expression of cell potency elicited by various environmental factors, no difficulties are encountered in allowing for the gradualistic type of change in many cells at the same time. That the degree of malignancy varies in different tumors is well known. This would be expected on the basis of tangential differentiation, for it is clear not only that tissue cells have different pros-

pective potencies because of the channels of differentiation into which they have already entered but also that the germ cells of different species and the tumor cells of the different tumor types have different prospective potencies.

According to the idea that cancer is a form of differentiation, benign and malignant tissues as well as normal tissues would be expected to show cell modulation. That tumor cells behave differently when grown in different localities, in different hosts (cf. Furth (*77*) for review), or in vitro has been well demonstrated. MacDowell (*78*) stated: "A cell with leukemic properties in one animal, on transplantation into another animal with a different genetic constitution, may behave as a normal cell." Such a host is regarded as nonsusceptible, which means that it does not provide the environment required by the given cell for the manifestation of leukemic properties. In MacDowell's words: "Leukemia results from certain cells in certain environments."

The tissue responses to scharlach R and sudan III, which have confounded the proponents of sudden changes, fit smoothly into the concept of cancer as tangential differentiation involving cell modulation. In the development of squamous-cell carcinoma, for example, epithelial downgrowth continues only as long as the agents are applied and disappears when the treatments are discontinued. This pseudo-malignancy therefore appears to be a form of cell modulation extending on one side of the indefinite line separating malignancy and normality while under the influence of the agent, and on the other side when not under this influence. In normal life, such processes as wound repair, pregnancy, glandular dysfunction, senescence, etc., involve a wide range of cell modulation and also cell maturation or differentiation. These processes, processes which involve cells, tissues, and organs in the most rigorous physiologic stress and strain, are the ones most often associated with malignancy. With the realization that malignancy is probably an inherent potentiality of every cell (which yet has residual competence), it seems a matter of sheer good fortune that most human beings manage to go through life without experiencing neoplastic changes in some form or other.

GENERAL VIEWS

From what has been said, there appear to be three general views of the underlying neoplastic principle. The first involves the idea that something comes into the cell from the outside, the second that certain of the inherent constitutional elements of the cell are changed, and the third that a process of development is involved. The first is identified with the virus idea of cancer, the second with somatic mutation, and the third with differentiation. At first sight these views seem to involve quite different approaches to the question of malignancy. However, when the particular aspects are taken into account in each case, it is found that they resolve very nearly into one and the same consideration.

Supporters of the virus view of cancer first regarded carcinogenesis as a type of infection and presumed that the self-perpetuating virus agent, which is carried along from cell to cell by transmission, must be continuously present in order to impel the invaded host cells to behave in a malignant fashion. As Rous has pointed out, however, a fair body of evidence indicates that this is not the case. Malignancy, once started, appears to be self-sustaining (without virus) just as physiologic states arising from differentiation are self-sustaining. There appears then no need for considering viruses as different from other chemical activators. The fact that a virus agent has the property of autosynthesis may be regarded as incidental and having no greater significance than the fact that urethane can induce anesthesia. Since it has been shown that tumors may be induced with physical agents and that the addition of exogenous substances is not always essential for their induction,

proponents of the virus theory have postulated "resident viruses" which are said to pervade all cells. Such a hypothetical agent is described as lying dormant in cells until stimulated by exogenous carcinogenic agents, at which time the viruses are said to mutate and to elicit the malignant response in cells. Since virus agents have not always been found in tumors induced by viruses, it has been more tenuously postulated that "dead end" viruses are involved, agents which are necessary only for the initiation of neoplasia. If one is forced to the view of resident or dead-end viruses, the idea that viruses comprise the underlying neoplastic principle loses its significance, and virus agents become relegated to the category of other activating agents and may be considered in the same light.

Mutations in the form of gene or chromosome alteration are believed to take place suddenly and completely when they occur. This has in fact been found to be true in the case of X-ray-induced mutations (Sax (*79*) and Kaufmann (*80*)). Furthermore, mutations both natural and induced have been observed to occur at random among cells and to vary widely in character in different cells. Thus, since neoplasia takes place progressively in groups of cells simultaneously, somatic mutation as known at present is not a satisfactory explanation of neoplasia. In order to utilize the mutation idea, one must postulate "selective" mutation. The idea that certain chemical agents may have a selective action on the inherent potencies of cells, however, is not tenable. In fact, the action of organizers in development appears to be of just this type. Moreover, as has been pointed out, a close similarity exists between the manner of response of cells to organizers in development and the response of cells to actuating chemical agents in carcinogenesis. Earle's work (*81*) dealing with carcinogenesis in vitro indicates that the changes toward malignancy take place slowly and progressively in the same way in many cells simultaneously and further that the changes in cells may be carried beyond the condition of optimal malignancy. If then we permit the term "mutation" to connote selective action by specific chemical agents and to apply to irreversible changes in extrachromosomal as well as chromosomal constituents, mutation or selective mutation seems a plausible explanation of neoplasia. Mutation in this sense, however, cannot be considered as different from differentiation, as differentiation may take place by a process similar to or the same as selective mutation.

Speaking of cancer in terms of differentiation as outlined herein is not entirely new. Reimann (*82*) in an analysis of the carcinogenic process described cancer in terms of differentiation but, as already mentioned, regarded malignancy as an abnormal type of development involving "spare parts" cells. Foulds (*83*), using a somewhat different approach from that given here, nevertheless arrived at much the same conclusion. Gruenwald (*84*) urged that tumor potentialities might be more appropriately described in embryologic terms than in pathologic. Spencer and his co-workers (*85*, *86*) regarded the change to the malignant state as an "adjustment process," using this expression to cover both modulation and differentiation as described in the present paper.

It is realized, of course, that calling cancer a form of differentiation does not explain the mechanism of carcinogenesis, as the process of differentiation itself has not been explained. On the other hand, relating cancer to differentiation is not relating it to some hypothetical process, the existence of which is unknown. Differentiation is a well-ordered process about which a great deal is known. If our views have been cleared to the extent that cancer need not be regarded as an escape process, that the cancer cell is a cell governed by its constitutional make-up and its environment the same as any other cell, and that the cancer cell is not necessarily one that has been in-

vaded by an exogenous agent, then our thinking will have advanced a long way.

Should it be satisfactorily shown that cancer is a form of cellular differentiation, it is to be expected that malignant cells yet contain unexpressed potentialities, that by applying the proper stimuli these cells may be guided into still other channels of activity and away from autonomous growth, and thus that a new approach to therapy will be developed.

SUMMARY

A possible mechanism of carcinogenesis is set forth, based in large measure on findings with physical carcinogens. According to the view developed, cancer may be regarded as a form of accessory or tangential differentiation. In this light, carcinogenesis is considered to be an expression of residual cell potencies elicited by a variety of cell changes either occurring spontaneously or induced by stimuli from external sources.

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THE RESULTS OF HOMOPLASTIC, HETEROPLASTIC, AND XENOPLASTIC TRANSPLANTATION OF THE OPTIC VESICLE IN TRITURUS AND RANA[1,2]

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INTRODUCTION

The transplantation and reimplantation of the vertebrate eye has been of great interest to the experimental embryologist. Transplants have been performed homoplastically (between individuals of the same species), heteroplastically (between individuals of closely related species), and xenoplastically (between individuals of widely distant species) to study such problems as the origin and differentiation of the optic vesicle, the development and growth of parts of the eye, return of vision, development of structures associated with the eye, and regulation in growth of transplanted eyes.

The experiments of Lewis (*1*), in which the optic vesicles of *Rana palustris* and *R. sylvatica* were transplanted to the optic, otic, abdominal, or tail regions of other embryos and in which optic vesicles of *R. sylvatica* were transplanted to the region between the ear and the eye of *Amblystoma* embryos, clearly demonstrated the ability of the optic vesicle to undergo complete histologic differentiation even when transplanted to unusual positions or to foreign hosts. However, it is clear that when the optic vesicle is transplanted to a distantly related host, some incompatibility may exist which causes the graft to degenerate even though it has undergone a considerable degree of differentiation before the process of degeneration begins.

In order to test for possible host effects upon the growth of transplanted tissues, the optic vesicle with the overlying ectoderm of *Amblystoma punctatum*, a small, rapidly differentiating species, and that of *A. tigrinum*, a larger, more slowly differentiating species, were exchanged by Harrison (*2*). These experiments indicated that the difference in the growth rate in the two species depended upon something inherent in the cells of the species and not upon growth substances circulating in the body. The eye of the *punctatum* embryo retained its characteristic growth rate even when placed upon the body of the *tigrinum* embryo and vice versa.

In a series of xenoplastic combinations between several species of *Rana* and *Amblystoma*, Hewitt (*3*) confirmed the conclusion that transplanted optic vesicles are capable of complete self-differentiation although the optic nerves in these transplants failed to connect with the host brain. Certain characteristic abnormalities were present in the xenoplastically transplanted anuran eyes, e. g., the vitreous bodies were either small or lacking; the lens, under considerable pressure, was constricted or pushed forward out of the eye or backward into the eye. In the reciprocal combination, the urodelan grafts healed into place and appeared normal for 1 to 10 days; but within 7 days the grafted epidermis disappeared, and no eye appeared in the position where the graft was implanted. There thus appears to be some incompatibility between the urodelan and anuran tissues, which was evidenced when urodelan tissue was transplanted to an anuran body but not when the reverse graft was made.

Twitty and Schwind (*4*) tested inherent growth potentialities by means of heteroplastic transplantation of various organs. Optic vesicles (with lens ectoderm) were transplanted between *Amblystoma punctatum* and *A. tigrinum*. Measurements of the eyes indicated that the normal eye of the donor and

[1] Revision of a dissertation submitted to the Board of University Studies of The Johns Hopkins University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author wishes to express her gratitude to Dr. W. Gardner Lynn under whose direction these experiments were carried out.

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the transplanted eye were of the same size. This agreement was maintained during most of the larval period. At metamorphosis a difference arose; and at that time the normal *tigrinum* eye became slightly larger than the transplanted eye on the *punctatum* host, and the transplanted *punctatum* eye became larger than its control. With maximum feeding, these discrepancies disappeared, and the behavior of the heteroplastically transplanted organs thus indicated that the factors controlling their growth were intrinsic to the grafted tissue. From a later series of experiments, Twitty (*5*) concluded that the potential size of the eye was determined largely by intrinsic factors, but that the expression or realization of this potentiality during the growth of the animal depended upon its interaction with a gradually changing organic environment.

Further studies on the relative growth of amphibian eyes were made by Twitty and Elliott (*6*) in a series of experiments in which optic vesicles with their attached lens ectoderm were transplanted between embryos of *Amblystoma tigrinum* and *Triturus torosus* in the tail-bud stage of development. Measurements of the grafted eyes indicated that the grafted *Amblystoma* eye, although exceeding the size of the normal *Triturus* eye, fell significantly below the size of the normal eye of the donor. This retardation was confined to the first 12 days of the experiment and was followed by partial recovery of the grafts. Further observations on larval eyes, transplanted between these two species, indicated that the *tigrinum* eyes achieved complete size regulation although the *torosus* eyes never quite attained full-size regulation.

The present experiments were undertaken with a view to studying the general growth processes in optic-vesicle transplants made between three species of the newt, *Triturus viridescens*, *T. pyrrhogaster*, and *T. torosus*, and the frog, *Rana pipiens*. The first series of experiments dealt with the results of homoplastic transplantation of the optic vesicle in these forms, the second with heteroplastic transplantation of the optic vesicle in two species of *Triturus*, and the third with attempts at xenoplastic transplantation between *Triturus* and *Rana*.

MATERIALS AND METHODS

Embryos of the three species of newts mentioned and of the frog were used in these experiments.

Right optic vesicles with their attached lens ectoderm were exchanged between members of the same species, of closely related species, and of distantly related species. Eggs were obtained by induced ovulation, according to the method described by Rugh (*7*). The operations were performed in the tail-bud stage of development with finely ground surgical needles and hair loops. During and for an hour following the operation, the animals were kept in small dishes containing a 0.2-percent sodium chloride solution. Following the operation, the transplants were held securely in place by glass bridges until they became well-established. The animals were maintained for approximately 3 weeks postoperatively, and during this period measurements of the anteroposterior diameters of the eyes were made at regular intervals with the aid of an ocular micrometer, as described by Harrison (*2*). The animals were fixed in Bouin's fluid, and microscopic sections of the normal and transplanted eyes were made and were stained with hematoxylin and eosin.

OBSERVATIONS ON THE GROWTH AND DIFFERENTIATION OF TRANSPLANTED OPTIC VESICLES

HOMOPLASTIC TRANSPLANTS

Optic vesicles of *Triturus pyrrhogaster* were transplanted homoplastically and orthotopically at the tail-bud stage of development. They became established easily, and healing took place rapidly. As early as the second day after transplantation the lenses had formed in the transplanted optic vesicles and were apparent when the animals were examined. Differentiation of the

eye proceeded rather rapidly; and by 9 days after operation the iris was pigmented, and ocular movements were observed. The iris of the normal, unoperated eye became pigmented somewhat more rapidly than that of the transplanted eye, the pigment appearing in the normal eye about the end of the first week after operation. The anteroposterior diameters of the normal and transplanted eyes were measured at intervals. The results of the measurements obtained for one group of animals can be seen in figure 1. On the average the transplanted eyes did not attain the size of the normal host eyes during the experiments.

It is evident from figure 1 that the normal and transplanted eyes increased in size at about the same rate. The difference in the size of the normal host eyes and the transplanted eyes was greater at the beginning of the experiment than at the end, and one might expect that this difference would have been entirely obliterated had the animals been maintained for a longer period, until metamorphosis or longer. Nevertheless, it is significant for the later study of heteroplastic and xenoplastic combinations that the operation did retard the growth of the eyes even when the host and donor were of the same species.

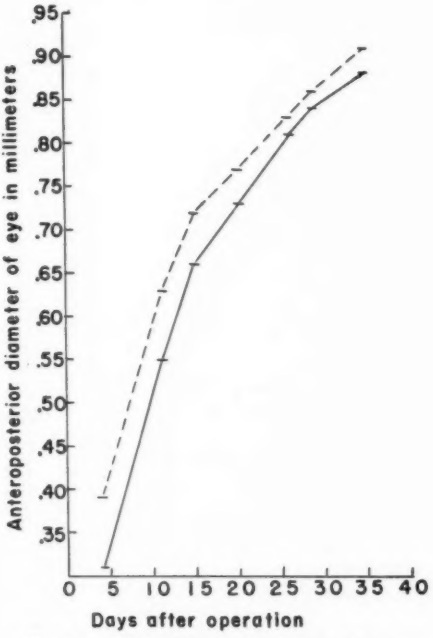

FIGURE 1.—Average rate of growth, over a period of 34 days, of transplanted (solid line) and normal (broken line) eyes of 7 specimens of *Triturus pyrrhogaster*.

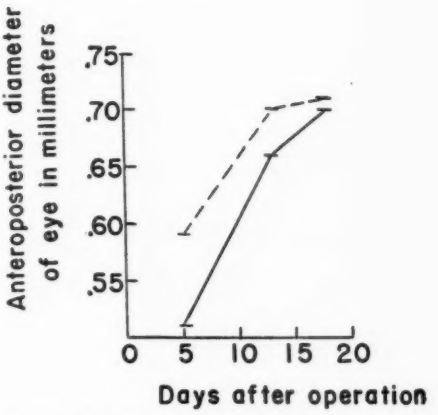

FIGURE 2.—Average rate of growth, over a period of 27 days, of transplanted (solid line) and normal (broken line) eyes of 7 specimens of *Rana pipiens*.

In exchanges of optic vesicles between *Rana pipiens* embryos, healing occurred very rapidly, and within 24 hours after operation, the transplanted optic vesicles were well-established. Differentiation of the lens and iris could be detected on the second day after operation, about the same time that it was observed in normal eyes. In about 5 to 7 days well-formed, normal-appearing eyes were present. Measurements of the anteroposterior diameters of the unoperated and the transplanted eyes were made at intervals. The results for one group are shown in figure 2. As in the previous experiment, the transplanted eyes did not reach the size of the normal host eyes during the period that they were under observation. The results are in accord with those obtained in the *pyrrhogaster* transplants.

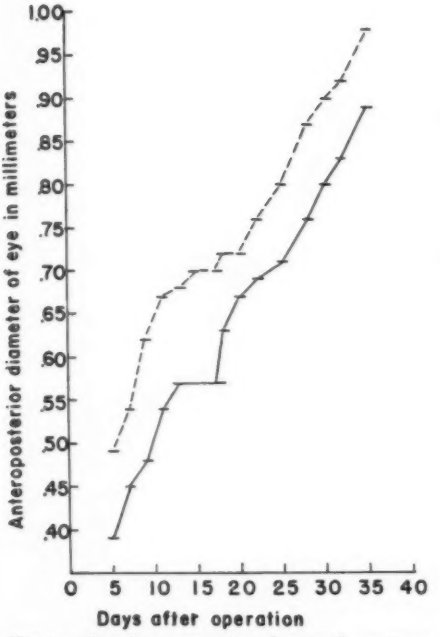

FIGURE 3.—Average rate of growth, over a period of 64 days, of transplanted (solid line) and normal (broken line) eyes of 18 specimens of *Triturus viridescens*.

Results similar to those described for the *Triturus pyrrhogaster* and the *Rana pipiens* series were obtained in similar exchanges with *T. viridescens*. Transplanted optic vesicles became established the first day after operation, and differentiation began on the second day. The anteroposterior diameters of the normal and the transplanted eyes for one group in this experiment are shown in figure 3.

HETEROPLASTIC TRANSPLANTS

Optic vesicles of *Triturus pyrrhogaster* when implanted orthotopically in *T. viridescens* became established readily. The reverse combination was equally successful. The growth of the eyes of one group, as indicated by the measurements of their anteroposterior diameters, is shown in figure 4. It will be remembered that the eyes of *T. pyrrhogaster* are normally slightly larger than those of *T. viridescens*.

The transplantation of optic vesicles of *Triturus viridescens* to *T. pyrrhogaster* resulted in the formation of eyes which were retarded as compared with the host eyes and also as compared with the unoperated eyes of the donor. Moreover, the eyes of *T. viridescens* in *T. pyrrhogaster* were significantly retarded even as compared with the homoplastically transplanted eyes of *viridescens*. This would seem to be the crucial comparison, indicating a definite effect of the host upon the growth of the donor eye in this heteroplastic combination.

The reverse combination, *pyrrhogaster* eyes in *viridescens*, gave evidence of a similar effect. The *pyrrhogaster* eye was retarded in growth as compared with the normal donor eye, the normal host eye, and the homoplastically transplanted eye.

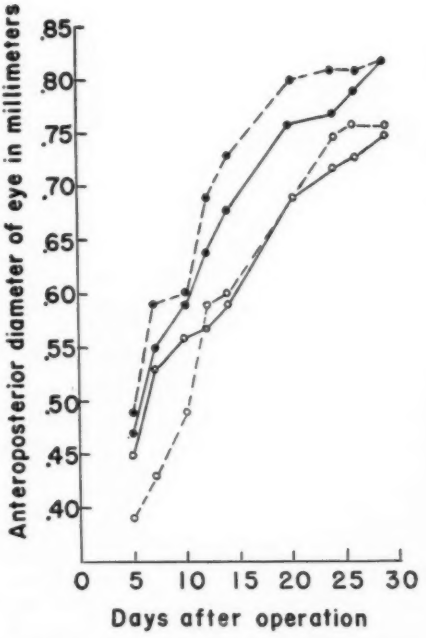

FIGURE 4.—Average rate of growth of transplanted (open circles) and normal (closed circles) eyes of *Triturus pyrrhogaster* (broken lines) and *T. viridescens* (solid lines) in heteroplastic combination.

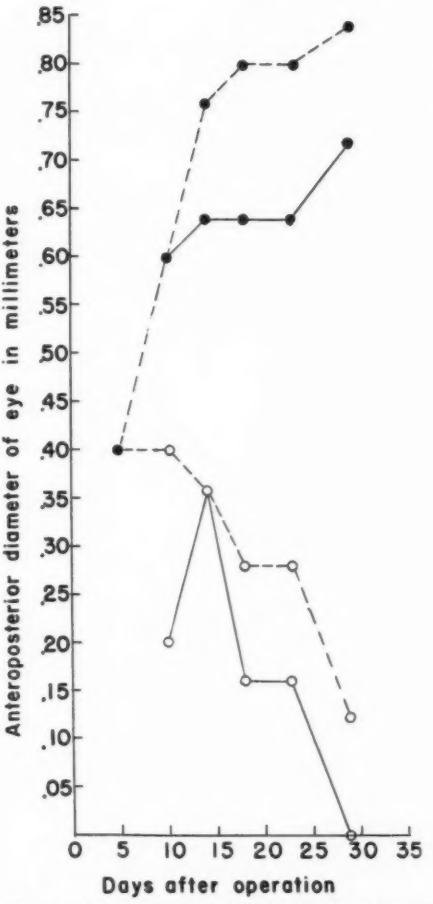

FIGURE 5.—Average rate of growth of transplanted (open circles) and normal (closed circles) eyes of 1 specimen of *Rana pipiens* (solid lines) and 1 specimen of *Triturus pyrrhogaster* (broken lines).

XENOPLASTIC TRANSPLANTS

Optic vesicles were exchanged xenoplastically between *Triturus pyrrhogaster* and *Rana pipiens*. The transplanted optic vesicles became established in both animals by the first day after operation, and healing occurred rapidly. Frequently the *pyrrhogaster* eyes were considerably reduced in size after transplantation. In some cases, differentiation began as early as the second day after operation, but in others not until some time later. Measurements of the anteroposterior diameters of the transplanted eyes revealed that in both combinations the transplanted eyes usually increased in size for a few days and then began to decrease and ultimately disappeared. Disappearance of *R. pipiens* eyes transplanted to *T. pyrrhogaster* occurred as early as 24 days after operation. Frequently the *pyrrhogaster* eyes transplanted to *pipiens* differentiated but did not become pigmented. In some cases the eye did not differentiate, and the transplanted tissue was present as an opaque mass.

One case (fig. 5) illustrates the events which occurred when reciprocal right eye transplants were made between *Triturus pyrrhogaster* and *Rana pipiens*. The normal left eye of each species increased in size throughout the experiment. The transplanted *T. pyrrhogaster* eye increased for 14 days and then decreased until it disappeared. The transplanted *R. pipiens* eye never increased but remained constant for 10 days after which it began to decrease.

Essentially the same results were obtained in exchanges between *Triturus torosus* and *Rana pipiens* and *T. viridescens* and *R. pipiens*. Optic vesicles which did become established underwent differentiation and an increase in size followed by a decrease with ultimate complete disappearance.

HISTOLOGIC STUDY OF THE TRANSPLANTED EYES

The results obtained for the three homoplastic groups were essentially the same. The transplanted optic vesicles differentiated normally in most cases. The condition of a homoplastically transplanted eye in *Triturus viridescens* 35 days after operation is shown in figure 6, *A*. The layers of the retina have differentiated fully, but they do not contain as many cell layers as do the layers of the retina of the normal unoperated eye. The lens and vitreous body are normal in appearance. In most transplants of this type, the optic nerve was present and connected with the brain of the host. In a few cases

the nerve did not connect with the brain of the host but frayed out in the surrounding tissue. In some instances there was some distortion of the retina, the layers being thrown into folds, and in others the pigment and sensory layers of the retina were absent or interrupted in certain areas, as shown in figure 6, *B*. In certain cases the number of cells in the layers of the retina was reduced. This was most apparent in the ganglion-cell layer. Occasionally the vitreous body was slightly reduced in size, and sometimes the lens was almost completely surrounded by the retina.

Histologic examination of the heteroplastically transplanted eyes revealed

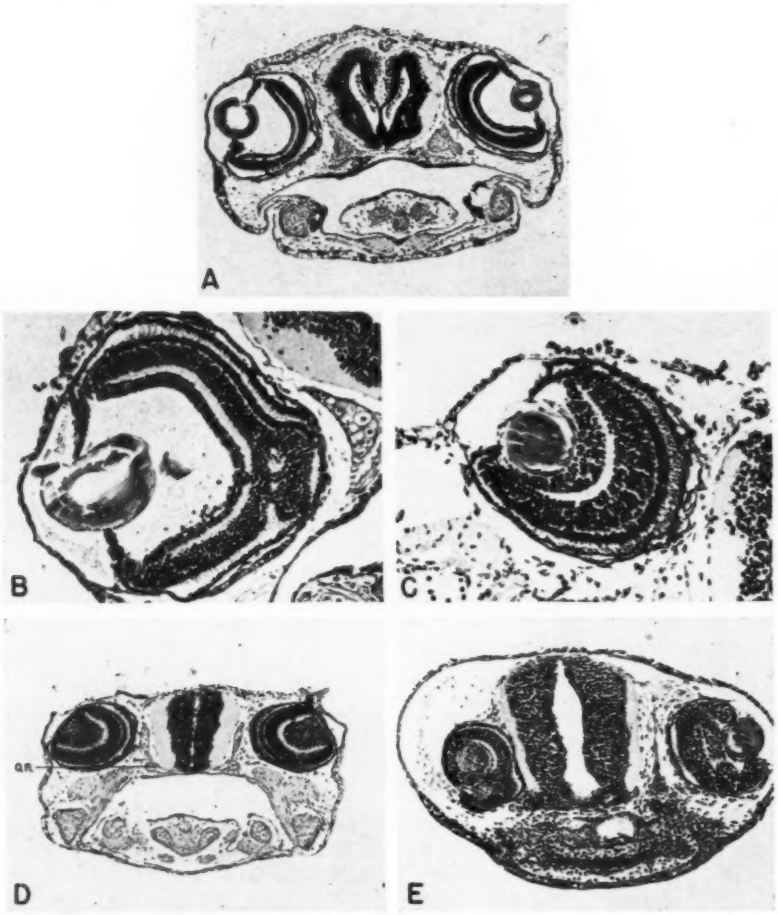

FIGURE 6.—*A*, A homoplastically transplanted eye (on the left) in *Triturus viridescens* 35 *T. viridescens* eye 35 days after operation. × 115. *C*, Absence of a vitreous body in a *T. pyrrhogaster* eye in *T. viridescens* 30 days after operation. × 115. *D*, Connection of the optic nerve of a *T. viridescens* eye (on the left) with the brain of *T. pyrrhogaster* 30 days after operation. o. n., optic nerve. × 40. *E*, Encapsulation of the lens of a *Rana pipiens* eye (on the left) in *T. pyrrhogaster* 6 days after operation. × 40.

that the conditions which existed in heteroplastic combinations were essentially the same as those in homoplastic combinations. The transplanted optic vesicles in both species usually differentiated normally, though, as has been seen, the eyes were smaller than the normal host eyes. In certain cases the retinae were distorted and the layers thrown into folds. In some cases the vitreous body was reduced in size, and in others it was almost entirely or completely lacking (fig. 6, *C*). When this condition existed, the lens was in direct contact with the ganglion-cell layer of the retina. In all the sections studied, the lens had differentiated fully, but occasionally the retinal layers were interrupted for short distances. Optic nerves usually formed in these transplants and in some cases were connected with the host brain. The connection of the optic nerve of the transplanted eye of *Triturus pyrrhogaster* with the brain of the host *T. viridescens* is illustrated in figure 6, D.

It was evident that the transplanted optic vesicles in xenoplastic combinations became established a short time after operation. In this respect they behaved just as did the transplanted optic vesicles in homoplastic and heteroplastic exchanges. However, in subsequent development they differed from these in numerous respects.

The results which were obtained in the *Rana pipiens* eyes transplanted to *Triturus pyrrhogaster* were essentially the same in all the sections examined. The transplanted tissue differentiated into retina and lens in nearly all cases, but occasionally the lens was absent. The retina was usually composed of its normal layers, but in practically all animals the layers were distorted in certain portions of the eye. In the majority of the animals maintained for longer than a week, when a lens was present, it was almost completely encapsulated by the retinal layers (fig. 6, *E*). In some cases the lens was normal, but usually it was reduced in size, distorted in outline, or vacuolated. The last-mentioned condition can be seen in a *pipiens* eye 17 days after operation in figure 7, *A*.

Frequently the transplanted eyes were set more deeply than the normal host eyes, and often they were adjacent to the brain of the host. This situation was found only in xenoplastic combinations.

Optic vesicles of *Triturus pyrrhogaster* when transplanted to *Rana pipiens* did not reach the degree of differentiation attained in the reverse combination. The *pyrrhogaster* optic vesicles became established a short time after transplantation and the retina and lens were formed. Figure 7, *B*, shows a transplanted *pyrrhogaster* eye 3 days after operation, in which the lens and retina have differentiated. The retina did not differentiate into its constituent layers in any of the transplanted eyes in this series. About 5 days after operation, the *pyrrhogaster* eyes usually appeared as masses of undifferentiated cells. Generally a lens remnant was present (fig. 7, *C*). In 50 percent of the animals preserved from 9 to 20 days after operation, the transplanted eye was present as a mass of undifferentiated cells, and in the other 50 percent, the transplanted eyes had disappeared entirely. In both the *Triturus pyrrhogaster* and *Rana pipiens* transplanted eyes, no optic nerves were ever observed to have been formed.

Transplanted *Rana pipiens* optic vesicles became established readily in *Triturus torosus* and underwent differentiation. The retina possessed its usual layers but was distorted in certain areas. A poorly organized structure which represented the undeveloped lens was usually present. In some cases the lens was entirely lacking (fig. 7, *D*). The transplanted eyes were frequently deeply set or adjacent to the brain and were smaller than the normal host eyes. Transplanted eyes were not found after 15 days in any of the *T. torosus* animals which were preserved.

Triturus torosus optic vesicles transplanted to *Rana pipiens* became established and underwent differentiation for a short period. In animals preserved a

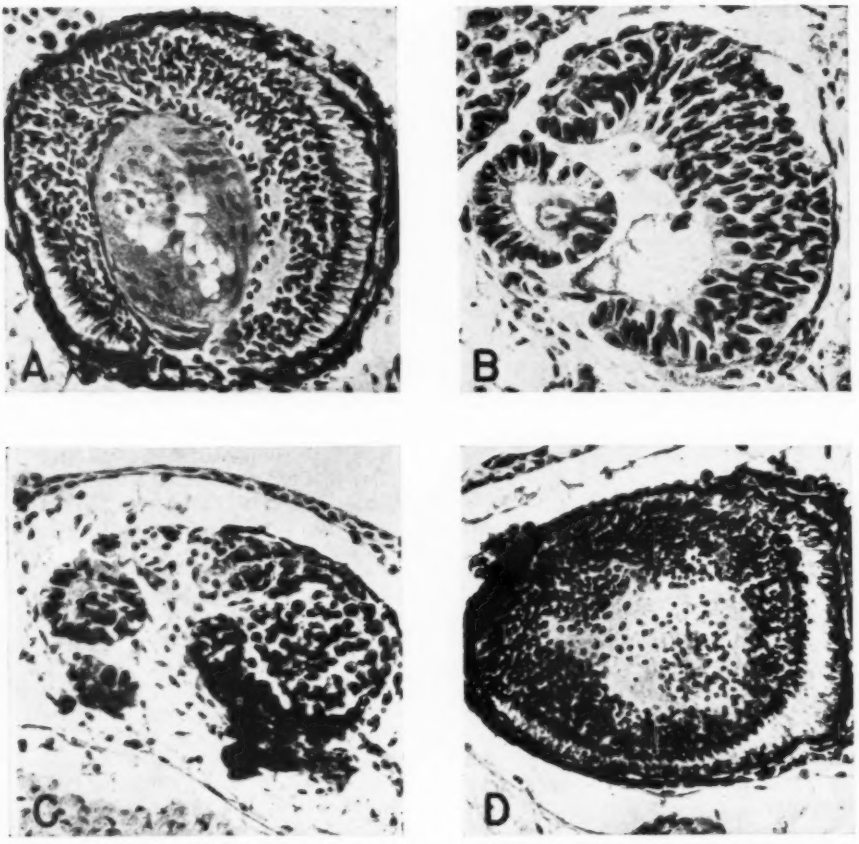

FIGURE 7.—*A*, Vacuolated condition of the lens in a *Rana pipiens* eye in *Triturus pyrrhogaster* 17 days after operation; *B*, Differentiation of retina and lens in a *T. pyrrhogaster* eye in *R. pipiens* 3 days after operation; *C*, *T. pyrrhogaster* eye in *R. pipiens* 8 days after operation, showing a mass of undifferentiated retinal cells, a small degenerate lens, and a mass of retinal pigment; *D*, *R. pipiens* eye in *T. torosus* 13 days after operation, showing the absence of a lens. × 175.

few days after operation, the transplanted eye was present as a mass of more or less undifferentiated tissue. A lens remnant was usually present but was imperfectly formed. The transplanted eyes were smaller than the normal host eyes. Frequently the only vestige of a transplanted eye was a mass of undifferentiated tissue. Transplanted *T. torosus* eyes were not found in sectioned *R. pipiens* specimens 9 days postoperatively. Optic nerves were not found in the transplanted eyes in any of the reciprocal exchanges.

DISCUSSION

The results of the present experiments are of interest in relation to these diverse problems: (1) Determination and self-differentiation; (2) inherent growth potentials in the tissues of different species; and (3) tissue incompatibility or antagonism in animals of more dis-

tantly related groups. These are fundamental problems in normal differentiation and growth and also in neoplasia.

With respect to the first, that of determination and self-differentiation, the present work adds further confirmation to the results of many previous investigators. At the tail-bud stage in all amphibia thus far investigated, the optic vesicle has complete powers of self-differentiation so that when it is transplanted to an unusual position or to a new host it is able to form an eye with all its characteristic parts, usually in their normal relation. This was demonstrated for *Rana* by Lewis (*1*) and has since been shown for *Bombinator* by Spemann (*8*) and for *Triton* and *Amblystoma* by Adelmann (*9–11*). Even when the graft is made to a species in which, because of some incompatibility, the eye is not able to persist, it is able to undergo more or less complete histodifferentiation before the degenerative processes which lead to its ultimate destruction have been started. Thus Lewis (*1*) found that the optic vesicle of *Rana sylvatica* when transplanted to *Amblystoma* was able to differentiate fully but showed definite indications of degeneration in 17 to 19 days after operation. Hewitt (*3*) reported a similar situation in the case of the reverse transplant. This type of response occurs not only in the transplantation of normal tissues but also has been reported in the transplantation of a neoplastic tissue. Briggs (*12*) transplanted kidney adenocarcinoma from adult frogs (*R. pipiens*) to tadpoles of the same species. The tumor was transplanted successfully into several different sites in the tadpole and presented the appearance which it normally does in the adult frog, although it later underwent regression in animals that were approaching metamorphosis.

It must, of course, be realized that although in the transplanted optic vesicle normal parts may be differentiated, various abnormal relations may be established. Distortion of the retinal layers, displacement of the lens, and failure of the optic nerve to connect with the host brain are common. In some cases these abnormalities are clearly the result of atypical relations to skeletal or other parts causing pressure on the eyeball or blocking the growth of the nerve. Often, no obvious abnormal relations which might account for the condition are detectable. In the experiments reported in this paper, all these conditions were found. However, in general, homoplastic transplants result in the development of eyes which are similar to unoperated eyes in appearance and have optic nerves normally connected with the brain. This is also true of heteroplastic transplants between *Triturus viridescens* and *T. pyrrhogaster*. Thus the situation in homoplastic and heteroplastic transplants in the present experiments is essentially the same. The high percentage of cases in which the optic nerve of the transplant established connections with the host brain is somewhat surprising, for Harrison (*2*), in his heteroplastic transplants between *Amblystoma punctatum* and *A. tigrinum*, found that the establishment of such connections was relatively rare; and Schwind (*14*), in homoplastic and heteroplastic transplants of eyes in three species of frogs (*Rana pipiens*, *R. sylvatica*, and *R. palustris*), encountered a similar situation. In Schwind's experiments, most of the homoplastic and heteroplastic grafts underwent differentiation, but the large majority showed degenerative changes either before or following metamorphosis. On the other hand, Stone and his coworkers (*15–20*) showed that eyes transplanted both homoplastically and heteroplastically in late larvae of *A. tigrinum* and *A. punctatum* became functional. This same situation exists in homoplastic eye transplants in adult specimens of *A. punctatum* and *T. viridescens*.

In the xenoplastic combinations between *Rana* and the various species of *Triturus*, although differentiation of the parts of the eye was usually accomplished, abnormal relation of parts was more frequent than in the case of homoplastic and heteroplastic exchanges. Moreover, the transplantation of optic

vesicles from *Triturus* to *Rana* resulted in greater abnormality than did the reverse operation. As has been shown, the optic vesicles of *R. pipiens* transplanted to *T. pyrrhogaster* became established readily and differentiated fairly normally. The transplanted tissue differentiated into retina and lens in nearly all cases. The retina was composed of its normal number of layers, but usually the layers were distorted in some region. The lens in some cases was reduced in size, irregular in outline, or vacuolated, and in almost all cases examined it was encapsulated by the retinal layers. These transplanted eyes were reduced in size and did not possess any optic nerve. In the reverse combination, *T. pyrrhogaster* eyes in *R. pipiens*, the *pyrrhogaster* eyes in no case reached the degree of differentiation attained by the *pipiens* eye in *pyrrhogaster*. A lens was usually formed, but the retina did not differentiate into its constituent layers. Here again, the optic nerve was not observed to have been formed. In about 5 days after the operation, the transplant could be seen simply as a mass of undifferentiated tissue which persisted in some cases for as long as 20 days In the *T. torosus-R. pipiens* combinations, similar results were obtained. However, in this case, no transplanted *torosus* eyes were present after 15 days. These unusual conditions, found so frequently in the xenoplastically transplanted optic vesicles, were doubtless ascribable to some antagonism between the host tissues and those of the graft. Insofar as the results bear upon the problem of self-differentiation of the optic vesicle, it is clear that even in xenoplastic grafts the ability of the vesicle to differentiate all its characteristic parts is clearly evidenced, although degenerative changes may intervene early enough to confuse the picture somewhat.

The problem of inherent growth potentials has been the subject of considerable work, especially by Twitty and his collaborators (*4–6*), and as it was pointed out earlier, the general conclusion which seems to be indicated by these investigations is that the growth of transplanted tissue is largely determined by factors within the transplant rather than by host influences. The clearest evidence for this is derived from the experiments in which the eye primordia were exchanged between *Amblystoma tigrinum* and *A. punctatum* (Harrison (*2*), Twitty and Schwind (*4*), Twitty (*5*), and Twitty and Elliott (*6*)). The eyes of the larger and faster growing species retained their characteristic growth rate when transplanted to the body of the smaller and slower growing form, and vice versa. The experiments of Briggs (*12*) already mentioned, which involved the transplantation of a kidney adenocarcinoma from adult frogs to tadpoles, were conducted for the purpose of ascertaining whether the uncontrolled growth of the cancer cell was due to something within the cell itself or to the lack of a controlling mechanism in the host. The results indicated that the early larval environment did not exert any growth-controlling effect on the transplanted carcinoma. At the approach of metamorphosis, the implants underwent regression. In later experiments, Briggs and Grant (*13*) showed that regression of the implants occurred in nonmetamorphosing tadpoles (thyroidectomized and hypophysectomized) also, and they therefore concluded that it was not due to changes accompanying metamorphosis. Spindle-shaped mesenchyme cells and occasionally small, round cells accumulated around the implant, and it was thought that these might be responsible for the regression which took place.

In the present experiments, although the differences in growth rate were not nearly so great as in the *Amblystoma tigrinum* and *A. punctatum* combination, nevertheless differences in growth rate of the host and donor eyes were easily demonstrable. However, when the growth rate of the graft was compared with that of the normal donor eye, it was found to be considerably lower than the latter; and in fact the heteroplastically grafted eye grew at a lower rate than did the homoplastically

transplanted eye of the donor species. This would seem to indicate some effect of the host tissue upon the graft in the heteroplastic combinations. Such an effect, though not so strong as to cause degeneration of the graft, such as occurs in xenoplastic combinations, might conceivably be sufficient to bring about a temporary retardation in growth rate. This retardation probably was in fact only temporary since, as has been shown, the growth rates of the graft did approach those of the donor at later stages. In almost all the homoplastic and heteroplastic combinations in the present experiments, the transplanted eyes were smaller than the unoperated host eyes at the conclusion of the experiments. However, the difference in size of the two eyes decreased in most cases during the course of the experiments. It seems logical to conclude that this difference might have been entirely obliterated had the animals been maintained for longer periods, for instance until the time of metamorphosis or longer, but it is important to note that the operation does retard the growth of the eye even when the host and donor are of the same species.

The final problem is that of tissue incompatibility or antagonism. Xenoplastic transplants between *Triturus* and *Rana* resulted finally in degeneration and complete disappearance of the transplanted tissue. In the case of the *Rana* vesicles transplanted to *Triturus*, the grafts persisted for varying lengths of time but showed clear indications of degenerative changes within 20 days, whereas in the reverse combination persistence of the graft as an organized structure was only about 5 days. Such results had been reported before for other combinations. Lewis (*1*) reported degeneration of grafts in eye transplants between *Rana sylvatica* and *Amblystoma*, and Hewitt (*3*) in her work (transplantation of optic vesicles of urodeles to anurans in the following combinations, from *Amblystoma punctatum* to *Rana sylvatica*, *R. palustris*, and *R. sphenocephala*, and from *Axolotl* to *R. palustris*) showed a similar situation. In the present investigation, an attempt was made to work out the history of these degenerative changes by histologic examination of material fixed at various intervals after the transplantations were made. The general results of this phase may be stated as follows: *R. pipiens* optic vesicles transplanted to *Triturus pyrrhogaster* and *T. torosus* behave similarly. The vesicles become established and undergo differentiation, and retina and lens are formed. In nearly all cases, the lens is encapsulated by the retinal layers and is frequently abnormal, being vacuolated, reduced in size, or irregular in outline. Later, the transplants may degenerate and disappear. In the reverse combination, the degree of differentiation is not so high as in the combination mentioned. The graft differentiates into retina and lens, and these persist as such for a short time, but after approximately a week the graft is represented merely by a mass of undifferentiated cells.

The problem of tissue incompatibility or antagonism is important in tumor transplantation. As an explanation of susceptibility to tumor implants, Little and Strong (*21*, *22*) formulated a genetic theory of transplantation which is as follows:

> The fate of the implanted tumor tissue when placed in a given individual (host) is brought about by a reaction between the host, determined to a large extent by its genetic constitution, and the transplanted tumor cell, controlled to some extent by its genetic constitution.

Gorer (*23*, *24*) advanced an antigenic explanation for the fate of tumor transplants and suggested a rewording of the genetic theory of Little and Strong to include the action of isoantigenic factors genetically determined. Whatever the ultimate explanation may be, it is reasonable to suspect that the basic factors affecting incompatibility of both normal and neoplastic transplants may be similar.

SUMMARY

Further confirmation is added to the results of previous investigators, and

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some new evidence is presented concerning the situation that exists in homoplastic, heteroplastic, and xenoplastic optic-vesicle transplants. At the tail-bud stage, the optic vesicles of *Triturus viridescens*, *T. pyrrhogaster*, *T. torosus*, and *Rana pipiens* were found to have complete powers of self-differentiation, so that when they were transplanted to a new host, they were able to form eyes with all their characteristic parts, usually in their normal relations. Although the transplanted optic vesicles might differentiate their normal parts, various abnormal relations might be established. Distortion of the retinal layers, displacement of the lens, and failure of the optic nerve to connect with the host brain were common.

Both homoplastic and heteroplastic transplants generally resulted in eyes normal in appearance, with optic nerves connecting with the host brain. In almost all cases of homoplastic and heteroplastic combinations, the transplanted eyes were smaller than the unoperated host eyes at the conclusion of the experiments. The difference in size of the two eyes decreased in most cases during the experiments.

In xenoplastic combinations, although differentiation of the parts of the eye was usually accomplished, abnormal relation of parts was more frequent than in homoplastic and heteroplastic combinations. In xenoplastic combinations the transplanted tissue differentiated into retina and lens but underwent degeneration and in some cases disappeared entirely. This degeneration was more pronounced in transplants of urodelan tissue to anurans than in the reverse combination. Moreover, transplantation of urodelan optic vesicles to anurans resulted in greater abnormality than did the reverse operation.

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CHANGES IN THE ORGANS OF FEMALE C3H MICE RECEIVING THIOUREA

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After giving 0.25 percent thiouracil to young rats in the drinking water for 5 to 90 days, Williams et al.[1] reported that there were no notable alterations which could be attributed to this material, with the exception of those seen in the thyroid gland. The present paper reports observations after prolonged feeding of another goitrogenic compound (thiourea) to adult female mice susceptible to the development of spontaneous mammary tumors. The period of administration of the drug was much longer than in most of the previously reported experiments with such compounds. The structural changes observed in this strain of mice are definite and tend to become more pronounced with continued treatment.

Forty-six 11-month-old virgin female C3H mice without mammary tumors were placed on stock diet 77.[2] Twenty-one of these mice received this diet plus 0.25 percent thiourea mixed in the food. After 13 weeks, the percentage of thiourea was increased to 0.375. Table 1 shows that 0.25 percent thiourea in the diet caused no decrease in weight or food intake. Following the increase in the thiourea content of the diet, there was a very slight decrease in food intake and in body weight, which was, however, not statistically significant. Williams et al.[3] reported that thiouracil in the drinking water of young rats retarded growth, the effect becoming noticeable within 10 days.

TABLE 1.—*Average body weight and average daily food intake, for indicated week, of adult female C3H mice on control stock diet 77 and stock diet containing thiourea*

Mice	Week of experiment	Number of mice	Average body weight	Average daily food intake
			Grams	*Grams*
Control	0	25	25.3	0
Treated	0	21	26.0	0
Control	Fifth	20	26.0	3.0
Treated	do	20	26.0	3.0
Control	Tenth	14	26.0	3.0
Treated	do	14	26.0	3.0
Control	Fifteenth	10	27.6	3.0
Treated	do	12	25.7	2.9
Control	Twentieth	5	27.3	3.0
Treated	do	11	26.0	2.8
Control	Thirtieth	2	23.5	2.4
Treated	do	6	23.7	2.6
Control	Fortieth	0	0	0
Treated	do	5	23.8	3.0

Both control and experimental animals were killed as mammary tumors developed at times varying from 3 to more than 45 weeks after treatment started. At necropsy, tumor tissue, ovaries, uteri, mammary glands, kidney, pancreas, spleen, heart, lymph nodes, thymus, liver, and thyroid were fixed in Zenker-formol. The adrenals and pituitary and occasionally thyroid tissue were fixed in Champy fluid, and the liver, mammary glands, and occasionally thyroid tissue were fixed in Regaud's solution.

On gross inspection at necropsy, all organs of the 18 control animals observed appeared normal in size and texture. In the 19 thiourea-treated animals examined, the thyroids showed obvious enlargement, as was expected. In addition, after 13 weeks of treatment the uteri showed considerable decrease in size, and the ovaries were found regularly to be smaller than those of the controls. On microscopic examination after 7 weeks of treatment, red blood corpuscles were frequently found within some of the thyroid follicles, and at about this time a granular, greenish-yellow pigment appeared in the cytoplasm in the distal part of the cells lining the follicles (fig. 1). While the thyroids of all treated animals showed the expected hyperplasia, in no instance was there any evidence of neoplasia. Histologic examination showed little change from normal in the uteri

[1] WILLIAMS, R. H., WEINGLASS, A. R., BISSELL, G. W., and PETERS, J. B.: Anatomical effects of thiouracil. Endocrinology, 34: 317–328 (1944).

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[3] See footnote 1.

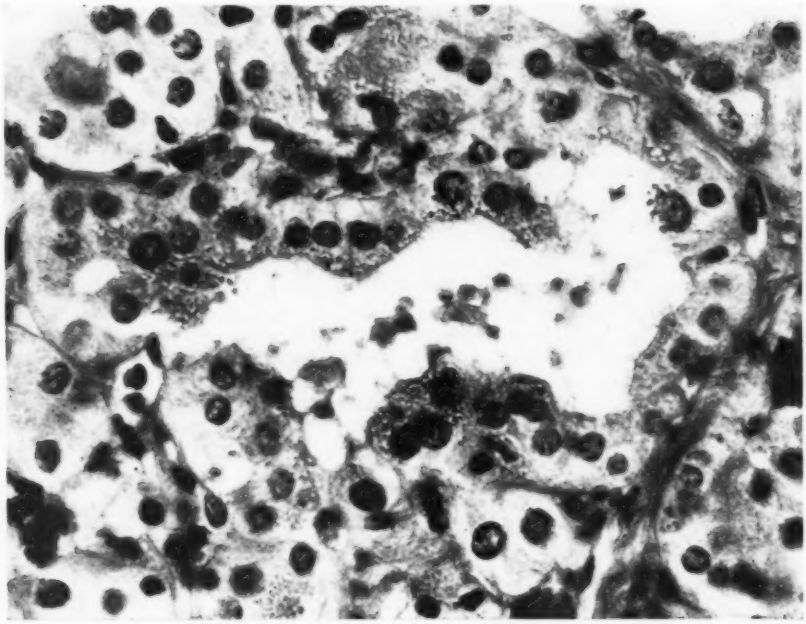

FIGURE 1.—A thyroid follicle of a 17-month-old female C3H mouse treated with thiourea for 25 weeks. The partially collapsed lumen contains several red blood corpuscles but little colloid. Pigment is visible in the distal part of many of the tall, columnar cells lining the follicle. The follicles of the normal gland are distended with colloid and are lined with cuboidal cells. Zenker-formol fixation; hematoxylin and eosin stain. ×1200.

although the changes associated with estrus were seldom encountered.

Large, fully mature follicles were only occasionally found in the ovaries, and when present they frequently showed degenerative changes in the granulosa cells. The majority of the ova appeared degenerate (fig. 2). The adrenals showed an obvious decrease in osmiophilic material, particularly in animals treated for more than 13 weeks. The width of the cortex also was less than that of the controls (fig. 3).

Further studies with younger animals and the use of thiouracil in place of thiourea are now in progress.

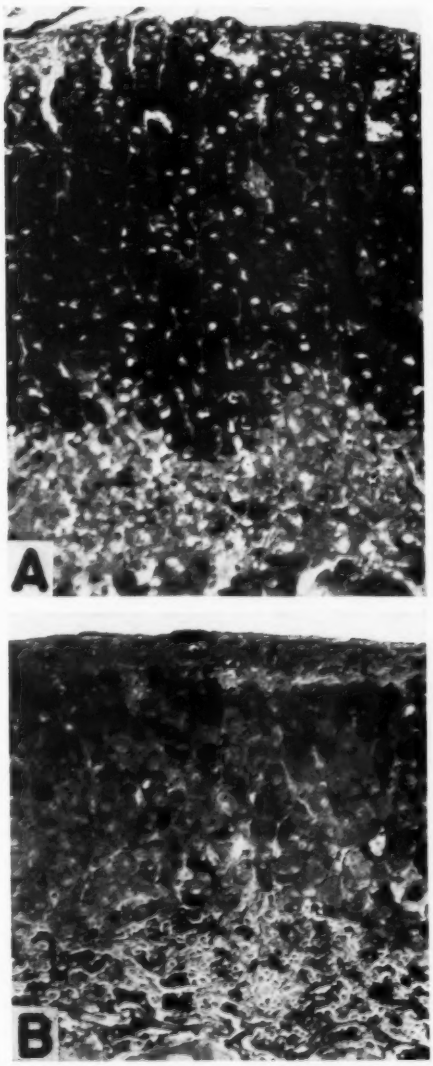

FIGURE 2.—*A*, Normal ovary from a 12-month-old female C3H mouse; *B*, Ovary from a 14-month-old female C3H mouse treated with thiourea for 13 weeks. Corpora lutea are numerous, but follicles are few and small. The majority of the ova are degenerate. Zenker-formol fixation; hematoxylin and eosin stain. ×42.

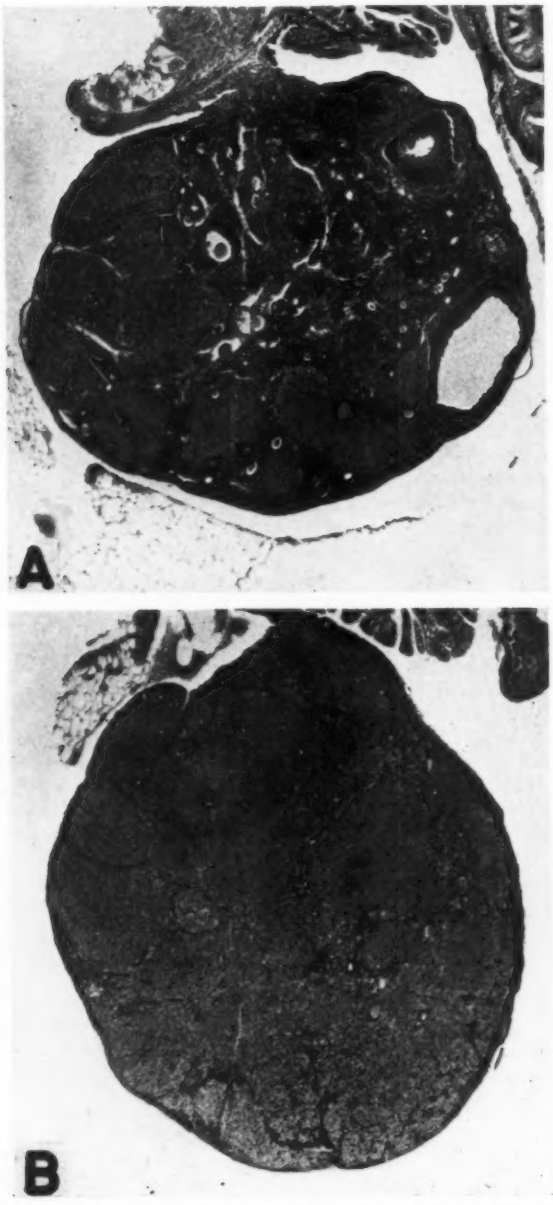

FIGURE 3.—*A*, Normal adrenal cortex from a 12-month-old female C3H mouse; *B*, Adrenal cortex from a 17-month-old female C3H mouse treated with thiourea for 25 weeks. The width of the cortex is less than that of the control, and fewer of the cortical cells contain lipid material. Champy fixation; osmic acid impregnation. ×195.

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